THE HOST-PATHOGEN RELATIONSHIP BETWEEN APPLE AND TRAMETES VERSICOLOR

AND FACTORS AFFECTING HOST SUSCEPTIBILITY.

by

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge contains no copy or paraphrase of material previously published or written by any other person except where due reference is made in the text of the thesis.

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Summary

Examination of a large number of apple trees infected with Trametes versicolor, indicated that the fungus was a facultative parasite which caused a white rot of the sapwood. Papery bark was a distinctive but non-specific external symptom of the disease. The development of papery bark was influenced by seasonal and nutritional conditions and was not a reliable indicator of susceptibility of trees to T. versicolor.

Discoloration of the sapwood always occurred in advance of fungal colonisation and decay. The formation of discolored wood involved the loss of starch and nuclei from ray and xylem parenchyma and the eventual necrosis of these cells. Necrotic cells were filled with a brown amorphous material which appeared to be oxidised phenolics. In young wood, ray and xylem parenchyma formed polysaccharide wound gum which was deposited in the vessels of the sapwood-discolored wood transition zone. The levels of K, Ca, Mg, moisture, pH, neutral solvent extractable materials and phenolics in discolored wood, differed from those in the adjacent sapwood. Bacteria were present in discolored wood in advance of the fungus.

Discoloration was a non-specific response of the sapwood to mechanical or pathological stimuli. In vitro decay tests indicated that discolored wood was more resistant to decay than normal sapwood. Discoloration of the wood and gum formation therefore appeared to be an active host resistance mechanism to fungal penetration and decay. The host reaction to wounding was related to the physiological cycle of the tree and with age cells lost the ability to synthesise wound gum and

reduced amounts of extraneous material were formed in the ray and xylem parenchyma. Evidence indicated that the resistance of young apple sapwood to colonisation and decay by T. versicolor was due to its vital nature and ability to elaborate an effective fungal inhibitory barrier. The susceptibility of living wood to fungal decay increased with age, due to a natural decline in host resistance.

The effects of nitrogen and phosphorus nutrition and water stress, on the susceptibility of young apple trees to T. versicolor were studied. Nitrogen deficiency decreased the susceptibility of trees as measured by the extent of papery bark and length of internal penetration and decay. Moderate phosphorus deficiency increased the extent of papery bark, but did not increase the length of fungal penetration and wood decay. None of the treatments significantly affected the host reaction to wounding or fungal invasion.

From a study of the host reaction per se and factors affecting susceptibility of trees to T. versicolor, it appeared that ageing was the dominant factor increasing the susceptibility of trees to the disease. However, the effects of other factors which may influence susceptibility can be explained by their influence on the fungal nutritional environment in the wood, or by their direct or indirect effects on host resistance.

Introduction

Trametes versicolor is generally regarded as a saprophyte but it can occur as a parasite on apple trees. In Tasmania, it is the only important pathogen which attacks the structural parts of apple trees. Its major significance is related to the white wood rot it causes in mature and re-worked apple trees.

T. versicolor has only been reported as an important parasite of living apple trees under Australian conditions. The reason for this is unknown. There are no apparent relationships between the incidence of the disease and such factors as variety or site, although it has been suggested that nutritional or cultural factors which cause a decline in tree vigour or decrease the reserve carbohydrates in the wood, increase the susceptibility of field trees to T. versicolor.

Previous studies on T. versicolor in apple have given attention to factors affecting host susceptibility but little study has been undertaken of the host-pathogen relationship. There is also a lack of basic knowledge concerning the physiology of the host in relation to fungal attack.

The objectives of this investigation were to examine the parasitic status of T. versicolor on apple and to conduct a study of the host-pathogen relationship, including an examination of the host reaction to fungal invasion. In addition, it was decided to examine the susceptibility of young apple trees to T. versicolor in relation to a number of factors thought likely to influence the susceptibility of field trees to the disease. It was hoped to integrate information from these studies to provide a hypothesis which could be used to explain variations in susceptibility of trees to T. versicolor.

LITERATURE REVIEW

NOTE: (1)   In this thesis the name <u>Trametes</u> <u>versicolor</u> (Linnaeus

ex Fries) Lloyd was adopted after Cunningham (1965). The

synonyms most frequently used for this fungus by other

authors include <u>Polyporus</u> <u>versicolor</u> L. ex Fr., <u>Polystictus</u>

<u>versicolor</u> (L. ex Fr.) Fr., and <u>Coriolus</u> <u>versicolor</u> (L.

ex Fr.) Quel. Cheung (1969) confirmed Cunningham's

classification of this fungus in the genus <u>Trametes</u>.

(2)   Literature reviewed has mainly concerned Angiosperm

(hardwood) trees. Literature dealing with Gymnosperm

(softwood) trees was only cited when of special interest.

Attack of living Angiosperm (Hardwood) trees by wood rotting fungi
with special reference to fruit trees.

Wood rotting fungi belonging to many genera of Basidiomycetes
attack living hardwood trees, resulting in economic and aesthetic
loss through the destruction of timber, fruit and ornamental trees.
Wood rotting fungi of both the brown and white rot types cause
sapwood, heartwood and root rots. Extensive records of such attacks
are given by von Schrenk and Spaulding (1909), Weir (1923), Jørstad
(1948), Wagener and Davidson (1954), Cartwright and Findlay (1958),
Peace (1962), Bakshi and Singh (1970). Most species causing sap and
heartwood rots gain entry through wounds on the aerial portions of
trees.

In many hardwood trees a clear delineation occurs between
the inner dead heartwood and the outer living sapwood. In trees
possessing heartwood the most significant decays are generally those
of the heartwood. As the heartwood is dead, the fungi attacking it
are not strictly parasites (Cartwright and Findlay, 1958). These
authors have proposed the term 'pertophyte' to describe those fungi
that attack the dead parts of living trees. Indirectly these fungi
may eventually result in the death of the tree by bringing about its
collapse through the destruction of the heartwood on which the
mechanical strength of the tree depends (Cartwright and Findlay, 1958).

The number of species of wood rotting fungi possessing any
significant ability to attack sapwood is few compared with the
number causing heartwood rots. Many that do are normally saprophytes
or only weakly parasitic (von Schrenk and Spaulding, 1909; Peace,

1962), while other species, such as Stereum purpureum, are capable of
more active attack of living wood. Assessing the importance and
parasitic ability of many of the species which cause sapwood rots in
living trees is often complicated by inadequate records.

Wood rotting fungi have been frequently recorded as causing
sapwood and heartwood rots in fruit trees, the decays being mainly
of the white rot type. Surveying the records reveals that many of
the wood rotting fungi attacking fruit trees have been recorded as
species of the genera Polyporus, Polystictus and Trametes. Defining
consistent host-pathogen relationships from the literature, however,
is difficult due to the unstable classification of these genera.
Furthermore, few of these decays have been investigated in detail.

Weir (1923) recorded eleven Polystictus species attacking
the heart and sapwood of various trees. Several species caused
heart and sapwood rots in apple, plum, cherry, prune and peach.
Brooks (1925) and Smith (1930) recorded Polyporus adustus and
Trametes hispida, respectively, as wound parasites of apple,
apparently causing sapwood rots. In Minnesota, Eide and Christensen
(1940), identified ten species of wood rotting fungi attacking fruit
trees. Five were Trametes and Polyporus species including Polyporus
versicolor. From the descriptions and illustrations it appeared
they mainly caused sapwood rots. Jørstad (1948) recorded ten species
of Trametes and Polyporus which occurred on apple, pear, peach and
plum in Norway. Most lived saprophytically on dead parts of trees
but some were noted as being weakly parasitic. Polyporus versicolor
was described causing a sapwood rot (heartrot) in Asiatic chestnut
(Hirt and Lowe, 1945). Wormald (1955) recorded Polyporus adustus,

P. sulphureous and P. hispidis as wound parasites on apple. Blumer
and Nuesch (1962) described Polyporus versicolor causing a sapwood
rot in cherry and apple trees. Numerous records have been made of
Trametes (Polystictus) versicolor attacking apple trees in Australia
(Birmingham, 1936; Ward, 1957; Doepel, 1962; Darbyshire, 1967).
Doepel (1962, 1965) claimed wood rotting of apple trees by Trametes
versicolor was a secondary phenomenon associated with dieback due to
other causes. While not clearly stated, the studies of Darbyshire
(1967) and Wade (1968) indicated that Trametes versicolor acted as a
primary parasite, attacking the living sapwood. Other important
species causing sapwood rots in fruit trees include the Stereum
species, particularly Stereum purpureum (Brooks and Storey, 1923;
Brooks and Moore, 1926; Brooks and Brenchley, 1931; Brooks, 1936;
Gäumann, 1950; Grosclaude, 1960, 1961, 1962, 1964a, 1964b, 1967, 1968)
and Fomes pomaceous (Fisher, 1934; Jørstad, 1948; Cartwright and
Findlay, 1958). F. pomaceous is closely related to Fomes igniarius
the most important sapwood rotting fungus attacking a wide range of
North American forest hardwoods (Ohman and Kessler, 1964).

Numerous records exist, therefore, to confirm the attack
of living fruit trees by wood rotting fungi. Many appear only as
weak parasites, while others, particularly Trametes versicolor, Fomes
pomaceous and Stereum purpureum can be more strongly parasitic. The
literature is often confusing due to loose usage of terms and many
authors fail to differentiate between sapwood and heartwood rots.

Only a few of the sapwood rots that occur in fruit trees
have been examined in any detail. These include the decays caused by
Stereum purpureum in stonefruits (Brooks and Storey, 1923; Brooks

and Moore, 1926; Brooks and Brenchley, 1931; Brooks, 1936; Grosclaude, 1960, 1961, 1962, 1964a, 1964b, 1967, 1968), Fomes pomaceous in plum (Fisher, 1934) and Trametes versicolor in apple (Doepel, 1962; Blumer and Nuesch, 1962; Darbyshire, 1967; Wade, 1968). With the exception of S. purpureum where the field of research has been wider, most study has been directed towards the host-pathogen relationships.

Sapwood, heartwood and discolorations of living wood.

The term 'wood' refers to the total structural parts of a tree. Sapwood and heartwood are recognised as the two normal physiological states of wood.

The Committee on Nomenclature (1964) defined heartwood as 'The inner layers of wood which, in the growing tree, have ceased to contain living cells and in which the reserve materials (e.g. starch) have been removed or converted into heartwood substances. It is generally darker in color than sapwood, though not always clearly differentiated.' As Shigo (1967a) noted this definition is based on the major conditions of cell death, depletion of nutrients, deposition of materials in the cells and a minor condition, darkening of the tissue. The definition emphasises the non-functional nature of heart-wood as compared with the living, physiologically functional sapwood. Ziegler (1967) suggested the further subdivision of sapwood into wood which is still capable of conducting water and wood which has mainly a storage function.

Frey-Wyssling and Bosshard (1959) and Hillis (1962, 1968) have considered in detail the major anatomical, cytological, physical and

chemical changes associated with the transformation of sapwood into heartwood. In addition to those mentioned, major changes include degeneration of the cell nuclei, increases or decreases in moisture and gas content, increases in density and acidity and the impregnation of the wood cells with phenolic materials (heartwood substances). Conspicuous anatomical features of heartwood formation in many hardwood trees include the formation of tyloses or other extraneous materials in the former conducting elements. Tyloses (Committee on Nomenclature, 1964) are 'Outgrowths from adjacent ray or axial parenchyma cells through pit cavities in vessel walls, partially or completely blocking the vessel lumens.' The extraneous materials that can occur in heartwood include gums, resins and crystalline deposits (Hillis, 1962).

Not all tree species form heartwood (Busgen and Münch, 1929; Hillis, 1962), while in others the heartwood remains uncolored (e.g. spruce and linden) but otherwise identical with colored heartwood (Frey-Wyssling and Bosshard, 1959).

Normally where heartwood occurs there is a restricted transition zone from living sapwood to heartwood. In some cases the differentiation is more diffuse and living parenchyma cells occur within the so-called heartwood (Good and Nelson, 1951).

The dynamics of heartwood formation are poorly understood. Variations occur in the ratio of sapwood to heartwood even within the same species. The age at which heartwood formation commences shows big differences between species, and it is not known whether the process of heartwood formation is a continuous one (Hillis, 1962).

Major theories relating to the initiation and maintenance of heartwood formation, have been discussed in detail by Jorgensen (1962), Hillis (1962, 1968), Stewart (1966) and Ziegler (1967). Briefly these are: (1) heartwood forms as a result of natural ageing of the wood, (2) heartwood is the result of fungal activity in the wood, (3) the loss of water and entry of air into the wood stimulates the formation of heartwood, and (4) heartwood formation is the result of an excretion process allowing the tree to store toxic metabolic products. Most evidence favours the view that heartwood formation in trees is a physiological process that occurs in response to internal stimuli and in the absence of micro-organisms.

Heartwood formation is a natural and consistent feature of the life cycle of many trees. Frequently discolorations of variable shape and extent occur in the sapwood of trees, independently of heartwood formation. While these discolored tissues develop basic heartwood properties (lack of living cells and reserve materials, accumulation of phenolics and differential color), and often have been considered as heartwood, they are now generally considered to be different in origin, properties and mode of formation from normal heartwood. As Shigo (1967a) indicated, the traditional definition of heartwood given by the Committee on Nomenclature (1964), fails to differentiate between heartwood and such formations.

Numerous terms have been used to refer to these discolorations. These include mineral streak, mineral stain, wound heartwood, pathological heartwood, false heartwood, protection wood, blackheart, brownheart, redheart, blue butt, wetwood and heartwood (Shigo, 1967a).

Additional terms noted include fungal heartwood, stain wood, traumatic heartwood and gummosis.

Many of the above conditions arise or are at least initiated by wounding with subsequent microbial activity. Wounds are defined as disruptions of the bark which expose the underlying wood to the atmosphere (Shigo, 1965, 1967a). Wounds therefore include pruning cuts, logging injuries, branch breakages, fire scars, animal and insect injury, etc. Shigo (1965) has suggested the name 'wound-initiated discolorations' for all discolorations resulting from wounding and decay processes in living trees. This separates such discolorations, formed in response to external stimuli, from colored wood formed by normal heartwood processes, initiated in the tree by internal stimuli. The relation of discoloration in sapwood caused by drought, freezing and vascular wilts to normal heartwood and wound-initiated discolorations requires further investigation. For convenience, the term 'wound response' will be used to describe collectively, the physiological changes that occur in sapwood around wounds and fungal decays. These are initially reactions of the sapwood, although the precise influence of external factors in stimulating the changes remains to be elucidated.

In all situations where the decay of sapwood by wood rotting fungi occurs in trees, the affected and healthy wood is separated by a zone of dark discolored tissue, produced in advance of the decay (Brooks and Storey, 1923; Fisher, 1934; Hirt and Lowe, 1945; Good and Nelson, 1951; Good et al., 1955; Cartwright and Findlay, 1958; Doepel, 1962; Blumer and Nuesch, 1962; Ohman and Kessler, 1964; Grosclaude, 1966b; Good et al., 1968; Tattar et al., 1971). As the

entry of the wood decay fungi in these cases resulted from wounding, such discolorations should be considered as wound-initiated discolorations, even though the continuing stimulus for the production of discolored tissue probably comes from the activities of the decay organism.

Differences between heartwood and wound-initiated discolorations.

Discolorations of living wood other than heartwood are a relatively common occurrence in trees. Shigo (1965, 1967a, 1967b) and Shigo and Sharon (1968, 1970) have emphasized the importance of micro-biological factors in the origin and continued development of discolorations resulting from wounding, as compared with heartwood formation.

The initial discolorations under wounds can occur in the absence of micro-organisms (Lorenz, 1944; Grosclaude, 1966b; Barčukova, 1967; Shigo, 1967a; Shigo and Sharon, 1968; Sucoff et al., 1967). The coloration is generally attributed to the oxidation of cellular phenols and their polymerisation to form pigments and discolored deposits in cells (Shigo, 1965; Sucoff et al., 1967). How far the process can develop in the absence of micro-organisms is not known, but once a wound occurs it is unlikely to remain sterile for any length of time.

Shigo (1967a), Shigo and Sharon (1968) and other workers (reviewed by Shigo, 1967b) showed that there was often a succession of micro-organisms in the discolored tissues underlying wounds. As a general principle, initial colonisation was by bacteria and

non-Hymenomycetes. The activities of these organisms, together with abiotic factors acted to produce discoloration. Later, Hymenomycetes could become established leading to wood decay. Shigo (1967a) stated 'Many factors affect the rate and extent of development of these processes. Development may reach a point and then abate. Discoloration precedes decay, but decay does not always follow discoloration.'

Most studies concerning properties of discolored tissue have been devoted to wound heartwoods, either as they occur naturally or as a result of deliberate physical wounding (usually with an increment borer). The properties of zones of discoloration surrounding fungal infections (pathological or fungal heartwood) and areas of insect injury have been considered to a lesser extent.

Hart (1963) reviewed the results of earlier studies of natural wound heartwood and wound heartwood resulting from increment borings. Most authors considered wound heartwood was similar to natural heartwood in that it was often of similar color, lacked living cells or reserve materials and contained increased amounts of phenolic extractives. Some however, noted differences in color, morphology of cellular depositions and decay resistance between wound heartwood and heartwood. Most studies lacked the support of sound analytical data.

Hart's (1963, 1965) work was more definitive. Morphological and chemical comparisons were made of the sapwood, heartwood and wound heartwood (induced by increment borer) of Quercus alba (white oak), Acer saccharinum (silver maple) and Juglans nigra (black walnut). The range of color intensity of wound heartwood was greater than normally found for heartwood. The extent and development of gums and tyloses was also dissimilar. Chemical differences between the wood types were

more pronounced. Wound heartwood was higher in moisture and ash and was more alkaline than either sapwood or heartwood. The amounts of water soluble (hot and cold) and alkali soluble (1% NaOH) materials were significantly lower for wound heartwood than for either sapwood or heartwood, and only in the case of silver maple wound heartwood were solubilities intermediate between those of sapwood and heartwood. Hart (1963) concluded 'Wound heartwood is morphologically similar to but chemically different from normal heartwood. It cannot be considered a precocious development of normal heartwood.' Further results to support this conclusion were obtained by Hart (1968) with Maclura pomifera (osage orange) and Robinia pseudoacacia (black locust). In addition Sachs et al. (1966) found that Quercus rubra (red oak) stain wood had a pH similar to that of the heartwood but lower than that of the sapwood. Specific gravity was higher than for normal heartwood and the stain areas contained larger amounts of extraneous materials in the ray cells and vessels.

Differences and similarities in the composition of extractives have been noted between normal heartwood and discolored wood of the same species. Jorgensen (1961) found that the composition of extractives in induced and normal heartwood of Pinus resinosa was similar. Hillis and Inoue (1968) showed considerable differences between the composition of extractives formed in injured sapwood, normal heartwood and Sirex noctilio (Hymenoptera, Siricidae) affected sapwood of Pinus radiata. The abnormal extractive iso-olivil was found in the wood of Prunus jamasakura attacked by Coriolus versicolor (Hasegawa and Shirato, 1959). Hossfeld et al. (1957) found the discolored zones surrounding fungal attack in

aspen wood contained a fluorescent extractive. Unfortunately, it was not determined whether this extractive was definitely absent from the heartwood of the same species.

The differences in properties between wound heartwood, heartwood and sapwood of the same species are reflected in the results of in vitro decay tests. Most tests conducted showed the decay resistance of wound heartwood differed from that of either the sapwood or heartwood (Hossfeld et al., 1957; McNabb et al., 1959; Hart and Johnson, 1970; Shigo and Sharon, 1970).

Thus there are differences in the origin and properties of normal heartwood and the discolorations produced as a result of external stimuli. While the factors influencing the production of discolored tissue and the properties of that discolored tissue have been investigated to some extent, little is known of the actual reactions involved in their formation. The work undertaken (Sucoff et al., 1967; Wardell and Hart, 1970a), indicated that the processes are similar to those which occur in the sapwood-heartwood transformation but are modified by differences in environment, level of cellular activity and time scale.

Gums and tyloses - origin and properties.

A common feature of both the heartwood and the discolored tissues around wounds and fungal decays are tyloses and gum deposits in the vessels.

Tyloses have a definite wall and initially contain a full array of cell organelles (Côté, 1967). A major stimulus for the

formation of tyloses is the entry of air into conducting elements contiguous with ray cells (Chattaway, 1949). The development of tyloses depends on the dimensions of the pit aperture between ray cells and vessels. Species with pit apertures larger than 8-10μ form tyloses, while below this limit gum formation is predominant (Chattaway, 1949).

The production of gum is a well known plant phenomenon (Smith and Montgomery, 1959). The term 'gummosis' has been used since the nineteenth century to refer to the external exudation of such materials by plants. Species of Acacia and Prunus are particularly prolific gum producers. True plant gums are largely polysaccharide in nature (Smith and Montgomery, 1959).

As well as external exudation of gum, gum-like substances may be formed internally in the wood of trees. Two internal types of gum formation have been recognized. The first, common in members of the genus Prunus, is where gum is produced in well defined structures in the wood known as gum cavities or lacunae. Gum cavities are formed by the degeneration of the cells of the youngest wood (Butler, 1911). Later the cavities become incorporated in the older wood. The second type is the formation of gum in the vessel lumens without alteration of the cell arrangement. It has been suggested that gum formed internally in vessel lumens, differs from that formed in gum cavities or exuded externally. Grosclaude (1966a) considered the evidence insufficient. Although all these products are apparently polysaccharide in nature their chemistry is inadequately understood.

The formation of gum in vessel lumens in hardwood trees in response to injury, fungi and chemical poisons was established as a general phenomenon by Frank (1884, cited by Rhoads, 1917), Rhoads (1917), Higgins (1919). Frank was the first to use the term 'wound gum' to describe this material and the terms 'wound gum' or 'gum' have been used by many subsequent investigators including Rhoads (1917), Higgins (1919), Brooks and Storey (1923), Coster (1924), Swarbrick (1926), Brooks and Brenchley (1931), Willison (1932), Rankin (1933), Fisher (1934), Good and Nelson (1951), Good et al. (1955), Hart (1963, 1965) and Grosclaude (1966b). Many of these authors have used the term 'wound gum' in a dual context to refer to vessel lumen deposits and to depositions in xylem and ray parenchyma. While little is understood of the nature of the latter, indications are that these materials are probably polyphenolic in nature and as such are different to the polysaccharide gum. The term 'wound gum' should therefore be restricted to describe the colorless-brown polysaccharide material that can occur in vessel lumens in heartwood and discolored tissue. Gums produced by the metabolic activity of xylem and ray parenchyma cells (host-synthesised), must be distinguished from gels and gum resulting from the enzymic breakdown of host tissue (Talboys, 1968).

Two major theories have been proposed to account for the formation of wound gum –

(1) Munch (1910), cited by Rhoads (1917), proposed that gum arises after the death of living cells by the oxidation of the cell contents and their exudation into the vessels. Rhoads came to the same conclusion even though tyloses, which accompanied gum formation in some cases, were believed to arise from living cells. Although

significant, the work of these authors is confused and difficult to interpret.

(2) Prillieux (1875, cited by Willison, 1932) proposed that wound gum was formed by the living xylem cells from reserve materials (starch) and/or the pectic constituents of cell walls. Most early German authors supported the hypothesis (reviews by Rhoads, 1917; Higgins, 1919; Willison, 1932) as have many later workers (Brooks and Storey, 1923; Coster, 1924; Swarbrick, 1926; Willison, 1932; Fisher, 1934; Grosclaude, 1966b). Usually the only evidence advanced to support the hypothesis was the observation that disappearance of starch from the cells corresponded with the appearance of wound gum in the vessels. Higgins (1919) claimed wound gum was formed from pectic constituents of cell walls but no strong evidence to support this hypothesis has been advanced by later workers.

Chattaway (1949) and Talboys (1968) found that both tyloses and gum always originated from ray cells, or less frequently, from xylem parenchyma, prior to the death of the cells. Their illustrations of cells producing gum and tyloses, strongly support the conclusion and refute the claim of many authors that wound gum occurs within the lumens of xylem and ray parenchyma cells. Additional evidence that gum and tyloses are products of living cells is that rapid killing of sapwood prevents their synthesis (Rhoads, 1917; Coster, 1924; Willison, 1932). While xylem and ray parenchyma cells synthesise gum, little is known of the cellular metabolism involved in the process.

From the results of early German studies (reviewed by Rhoads, 1917; Higgins, 1919; Willison, 1932) together with the results of

Rhoads (1917), Higgins (1919), Swarbrick (1926), Willison (1932) and Grosclaude (1966b), it is possible to summarise the known properties of wound gums occurring in a large number of plant genera.

Depending on age wound gums range in color from colorless to brown. Initially they are viscous fluids but they can dry and age to brittle solids. They are insoluble in hot and cold water, organic solvents and cold acids. They are soluble in hot nitric acid and, when fresh, in 1% NaOH. In contrast, gums exuded externally are water soluble.

Wound gums stain with ruthenium red which is possibly evidence that wound gums contain pectic material. As gums age they develop staining reaction with phloroglucinol. Rawlins and Takahashi (1952) claim this is due to the presence of aromatic aldehydes rather than to lignification.

There is general agreement that wound gum is polysaccharide in nature, although it has not been possible to determine this directly by extraction and determination of structure. The belief seems to be based on inferences drawn from the known composition and structures of gums exuded externally. Hough and Pridham (1959) found the gum formed in the bark and fruit of plum contained glucuronic acid, galactose, mannose, arabinose, xylose and rhamnose. Zitko et al. (1965, cited by Rosik et al., 1968) determined the composition and structure of apricot gum and found it was a complex polysaccharide based on glucuronic acid, mannose and galactose.

Wound gums probably contain extraneous materials. Early workers thought the gums become lignified and Hough and Pridham (1959) found a 'lignin-like substance' in external plum gum. Polyphenolic

materials may be present. Although many workers obtained negative results with the usual tannin reagents, this may have been due to the protective effect of the polysaccharide gum. Hough and Pridham (1959) found peroxidase and polyphendoxidase activity in plum gum. In the absence of oxygen, gum was found to remain colorless, which indicated that the browning of gum was due to the oxidation of phenolic compounds.

Differences exist in the quantity and morphology of wound gum deposits in heartwood and discolored tissue. Talboys (1968) suggested that there may be more than one type of wound gum. In plum, Talboys observed a granular viscous form, and a type that formed brittle, resin-like, hemispherical globules or meniscus-like occlusions across the vessels. Both types had similar staining reactions except that the granular type stained less intensely for phenolics. Talboys suggested that the different forms may arise at different stages of activity of the gum producing cells.

External factors influencing the wound response.

Wounding exposes sapwood to a new atmospheric and micro-biological environment. Evaluation of individual factors which influence the response (reaction) of living wood to these changed conditions has proved difficult. The abiotic factors of aeration and desiccation, and micro-organisms have received most attention in this regard.

Entry of air and desiccation are a constant result of the wounding of wood unless special precautions are taken to prevent

their influence. Summarising much of the early German work, Büsgen and Munch (1929) and Rankin (1933) concluded that the complementary factors of desiccation and access of air were important wound stimuli. In sterile cherry twigs in vitro, Higgins (1919) found that gum formation only occurred in atmospheres of relative humidity below saturation. Higgins suggested desiccation was an important stimulus to changing the activity of the living cells of the wood. Likewise the prevention of aeration and desiccation by sealing wounds in apple with airtight coverings, prevented the formation of discolored tissue and gum (Temme, 1885; Prael, 1888; cited by Baker, 1931). Aeration was the apparent stimulus for tylose formation (Chattaway, 1949) and she believed it also favoured gum formation. Jorgensen (1961) and Coutts (1969) observed the formation of pinosylvins in sapwood of Pinus sp. exposed to slow desiccation and aeration. Lyr (1967) reported rapid drying of wounds on Pinus sylvestris decreased or prevented wound heartwood formation. The coating of wounds with resin that occurred during the trees vegetative period, protected wounds against rapid drying and increased the formation of pinosylvins in the wound heartwood. Barčukova (1967) found wound response in Populus tremula, Alnus sp., Prunus padus and Quercus robur was accelerated by the enrichment of the air with oxygen or carbon dioxide but retarded by higher nitrogen levels. This evidence, together with that previously cited concerning the occurrence of discoloration under sterile conditions, indicates that both desiccation and aeration probably play a major role in the alteration of metabolism of the living wood cells.

Other physical factors of the environment can affect wound response. External temperature is one such factor. Higgins (1919) found gum formation only occurred in the range 10-60°C in cherry twigs in vitro. Jorgensen (1961) found 25°C the optimum temperature for the formation of phenolic extractives in Pinus resinosa sapwood exposed to slow desiccation. Extractives did not form above 35°C.

The physical trauma of wounding was claimed to be at least partially responsible for wound response by Büsgen and Münch (1929), Brooks (1936) and Grosclaude (1966b). How this trauma acted was not clarified. Busgen and Munch (1929) suggested wounding stimulated the production of wound hormones that control wound response. Other environmental factors probably influence wound response indirectly through their effect on the tree e.g. nutrition, but these have not been investigated.

The invasion of wounds by bacteria and fungi, both saprophytic and parasitic, has long been considered an important factor in stimulating discoloration of sapwood. A long held view of heartwood formation was that it was induced by fungal activity (Hillis, 1962). The statements of Rhoads (1917) and Büsgen and Münch (1929) indicated that most early workers appreciated the likely importance of fungi in the wound response process, although little direct experimental evidence on fungal stimulation was available.

Brooks and Storey (1923) thought discoloration and gum formation associated with the invasion of plum wood by Stereum purpureum, was principally a host reaction to fungal invasion. Subsequently, Brooks (1936) considered that the pathogen was not

entirely responsible and that the initial response was due to wound

trauma. The magnitude and continuation of the response however, was

considered to be due to the pathogen. Grosclaude (1966b) was of a

similar opinion. Shigo's detailed review (Shigo, 1967b) of the role

of micro-organisms in discolorations and decay, showed that most

evidence supported the contention that micro-organisms aggravate dis-

colorations.

How micro-organisms stimulate discoloration remains far from

clear. Fungal culture filtrates stimulate wound response phenomena in

sapwood (Willison, 1932), and the metabolites produced by vascular

wilts have long been implicated in the cellular disorders and dis-

coloration induced by these diseases (Dimond, 1955; Beckman, 1966;

Talboys, 1968). No specific mechanisms are known by which wood rotting

fungi may influence discoloration. Thus, while it is generally

accepted micro-organisms are normally intimately involved in the dis-

coloration produced under wounds or around decays, precise experimental

evidence on their role and influence on discoloration processes is

lacking.

Function of wound response.

The two major views on the functions of the physiological

changes in wood around wounds and fungal decays embody the idea of

protection.

Many early workers (reviewed by Rhoads, 1917; Higgins, 1919)

thought that gum and tylose formation were a means by which the tree

re-established a closed system to protect the underlying wood from atmospheric influence. According to Rankin (1933) the loss of water and entry of air was prevented. Swarbrick (1926) showed wound gum very effectively sealed xylem tissue below cut surfaces in young apple wood. It was not considered whether such effective sealing by gum occurred in older wood.

The protection of the living wood from invasion by micro-organisms has been regarded as the most important role of wound response. The early German workers often claimed this role (reviewed by Rhoads, 1917; Higgins, 1919), and the frequent use of such terms as protection or shield wood in their writings reflected this idea. Rhoads (1917) thought protective effects in such zones would come from the relative freedom of such tissues from air, the presence of toxic breakdown products and the lack of easily assimilated nutrients. Rankin (1933) concluded that the protective role of wound response and particularly that of gum and tylose formation to act as a barrier to fungal penetration, still required convincing experimental proof.

Gum and/or tylose formation are conspicuous features of the wound response in many hardwood species and as such have attracted attention as possible physio-chemical barriers against fungal invasion. Willison (1932) found both Cytospora sp. and Sclerotinia sp. penetrated wound gum in peach twigs, although Sclerotinia sp. only survived briefly in wound gum areas. It was concluded that discoloration and gum formation slowed down the rate but did not prevent penetration by Cytospora sp. On the other hand penetration by Sclerotinia sp. was prevented. Hepting and Blaisdell (1936) found that a gummy protection

zone, impervious to normal pathogenic species of sapwood rotting fungi, developed under fire scars in Liquidamber styraciflua (red gum). In vitro decay tests showed gummed wood to be much more decay resistant than normal sapwood and they attributed this protective effect largely to the formation of gum in the wood.

Gum formation has received greatest attention as a possible protective barrier, in relation to Stereum purpureum infection in stone fruits. Resistance of stone fruits to S. purpureum was correlated with the formation of gum barriers in the wood (Brooks and Storey, 1923; Brooks and Moore, 1926; Brooks and Brenchley, 1931; Brooks, 1936). The invasion of plum sapwood by S. purpureum was always preceded by dis-coloration and gum formation in the vessels. In resistant varieties, growth of the pathogen ceased after a time. Around the periphery of the invaded tissue, there was a narrow dark zone of gum, termed a 'gum barrier' by Brooks, because it appeared to prevent further spread of the fungus. Susceptible plum varieties lacked the ability to form gum barriers or could do so only at certain times of the year. Brooks' theory satisfactorily explained the varying degrees of susceptibility and resistance to S. purpureum observed in stone fruits. Brooks did not elaborate on the possible wider application of his theory to explain resistance/susceptibility of other hardwood species to wood rotting fungi.

According to Grosclaude (1968), the most significant objection to Brooks' theory was that raised by Grosjeahn (1955, cited by Grosclaude, 1968) who claimed gum barriers were not necessary to arrest the activity of S. purpureum. The fungus showed an annual

periodicity in its activity in the tree, and Grosjeahn claimed that the formation of gum barriers was a secondary phenomenon due to cessation of the growth of the parasite. That author postulated resistance to S. purpureum was under the control of some other unknown aspect of host metabolism. The pattern with S. purpureum is further complicated by the possibility that it can produce toxins (Brooks and Brenchley, 1931).

The protective role of tyloses has received less attention than that of gum formation. Evidence that they may be important in limiting decay and discoloration comes from work with vascular wilts where tyloses are of known significance in limiting disease spread (Beckman, 1966; Talboys, 1968). Brooks (1936) postulated that the thickening of tylose walls which sometimes occurred, helped to check the spread of fungi in vessels.

The differences in opinion on the effectiveness of gum and tyloses to act as physio-chemical barriers to micro-organisms therefore warrant more detailed examination. As Grosclaude (1968) indicated, it is difficult to obtain experimental evidence for such a protective role for gum and tylose formation.

Phenolic extractives or other compounds formed in discolored wood may contribute to the protective effect of such tissues against decay fungi. Heartwood contains phenolic compounds which are responsible for most of the durability characteristics of heartwood as timber. Discolored wood contains similar, but not always identical extractives to those found in heartwood of the same species. Little investigation of extractives from discolored zones in hardwood trees

or their effect on decay organisms, has been undertaken. Somers and Harrison (1967) found that tannins extracted from the discolored wood of apricots infected with Verticillium dahliae, strongly inhibited the germination of Verticillium spores. Palct (1967) found tannins extracted from apricot heartwood inhibited the growth of Trametes versicolor. With the conifers Pinus taeda and Picies abies, Shain (1967, 1971) provided evidence that the resistance of sapwood of these species to Fomes annosus, depended mainly on the inhibitory phenolic compounds (particularly pinosylvin and pinosylvin monomethylether), which accumulated in the discolored tissue in advance of the fungus.

The work of Good et al. (1968) is of importance when considering the possible protective properties of discolored tissues produced around sapwood decays. Good and Nelson (1951) and Good et al. (1955) studied the discoloration produced around decays of Acer saccharum (sugar maple) and concluded that overall the changes that occurred (wound gum formation, increase in pH, moisture, mineral content), probably had a protective effect against decay organisms. Good et al. (1968) differentiated several distinct zones around infections of Fomes igniarius in sugar maple. From the centre of an infection these were - (1) a central white rot zone of advanced decay, (2) a zone of incipient decay representing a transition from soft decayed wood to firm discolored wood, and (3) an outer zone of firm discolored wood. Measurement of the respiratory activity of these zones, showed maximum activity resided in the zones of incipient decay and sound discolored wood. The respiration in these zones was associated largely with Fungi Imperfecti and bacteria, rather than F. igniarius.

It was concluded that initial colonisation of the wood was by bacteria and Fungi Imperfecti which destroyed in advance, the likely protective effect of the discolored zone against F. igniarius (high moisture, high pH etc.), and allow later colonisation by F. igniarius. These results and those of Shigo and others (Shigo, 1967a, 1967b), indicate the effectiveness of discolored tissue in protection against the invasion of decay fungi, probably depends in many situations on other organisms (bacteria and non-Hymenomycetes), rather than the decay fungi themselves. Many exceptions no doubt occur, and Shigo (1967b) recorded examples of decay fungi which cannot compete with both host defences and other organisms, and can only colonise fresh wounds.

An indirect method of examining the possibly protective effects of discolored tissues is in vitro decay tests. The results of such tests have proved variable. Hossfield et al. (1957) found discolored aspen (Populus tremuloides) more resistant to decay than sapwood controls, while McNabb et al. (1959) found the decay resistance of Acer saccharinum (silver maple) wound heartwood to be intermediate between that of sapwood and heartwood. In preliminary work, Edgren (1959) cited by Hart (1963), found wound heartwood of silver maple to be more susceptible to decay than sapwood or heartwood. Hart (1964) found wound heartwoods of Juglans nigra (black walnut) and silver maple were less resistant to decay than the respective sapwoods. Hart and Johnson (1970) reported wound heartwoods of Quercus alba (white oak), Robinia pseudoacacia (black locust) and Maclura pomifera (osage orange) to be more resistant to decay than sapwood of the same species, but usually less resistant than heartwood. Shigo and Sharon (1970)

found discolored sugar maple more resistant to decay than other wood types. Results from this type of test have therefore not always indicated increased decay resistance in discolored wood, but differences in conditions of producing discolored tissue, decay testing and test fungi could account for some of the variation observed. Interpretation of this type of data requires care in view of the findings of Good et al. (1968), that the protective effect of discolored tissue may depend on species other than wood decay fungi in overcoming the inhibitory barriers. In pure culture experiments, wood is a non-responsive substrate and the decay fungi do not have to compete with a range of other organisms as they do in nature.

Discoloration of sapwood involves a wide range of reactions of variable magnitude depending on the interaction between the tree and the environment. The available evidence points to a protective function for such reactions. Shigo's (1967b) statement best summarises the situation, 'If the protective barriers were effective in every case, there would be little or no internal defect in trees. Conversely, if the protective barriers were never effective, there would be little or no sound wood in trees.'

Internal factors influencing the wound response and the susceptibility of sapwood in living trees to wood rotting fungi.

Garber (1956) proposed a nutrition-inhibition hypothesis of pathogenicity. According to this hypothesis two host environments, a nutritional and an inhibitory environment, directly affect the fate of

the pathogen. The nutritional environment may be adequate or inadequate to support the pathogen and the inhibitory environment effective or ineffective in suppressing the pathogen. Only when there is an adequate nutritional environment and an ineffective inhibitory environment is the pathogen successful. Any consideration of susceptibility must consider both environments and the factors affecting them. In living wood the elaboration and maintenance of the wound response (assumed to be the inhibitory environment) is affected by external factors (biotic and abiotic), and internal (tree) factors. External environmental factors act directly or indirectly by modification of the internal factors. While the nature of the wound response and the external factors influencing it are understood to some degree, little is known of the internal factors affecting the response.

Some information exists on the effects of the physiological state of the tree on wound response. Swarbrick (1926) studied wound healing in apple, plum and other species throughout the year. Effective blockings with wound gum below wound surfaces, only occurred during the spring and summer months. Autumn and winter wounds were not effectively blocked until the following spring, when the trees returned to full physiological activity. Brooks and Moore (1926) found gum barriers in plum were formed with much greater facility during the summer months and attributed this to the greater physiological activity of the trees at that time. Willison (1932) observed a similar situation with peaches and grapes. Higgins (1919) found that gum formation was not necessarily dependent on active growth. Fungal inoculations and chemical poisons resulted in external exudation of gum

and gum formation in the vessels of plum during the dormant period, though the response was less than in the same treatments in summer. It seems therefore, that a localised and specific stimulus can induce cellular activity, even during the dormant period. Lyr (1967) reported that the synthesis of pinosylvins, associated with wound heartwood formation in Pinus sylvestris, only occurred when the tree was physiologically active over the spring-autumn period. Increment borings in Quercus bicolor (swamp white oak) resulted in less extensive discoloration in autumn than in summer (Wardell and Hart, 1970a).

Age of wood influences response ability. Rhoads (1917), Swarbrick (1926) and Good et al. (1955) observed that older wood had less ability to form gum or was less intensely discolored than young wood. Brooks and Brenchley (1931) observed that gum formed more readily in the young tissue near the cambium than in the older wood. The typical v-shaped infection fronts in limbs infected with Stereum purpureum (greater penetration in the older wood), were attributed to the more rapid longitudinal penetration of the pathogen, combined with the lesser ability of the old wood to react and stop fungal penetration by forming gum barriers.

When the susceptibility/resistance of the sapwood of living trees to wood rotting fungi is considered two aspects must be noted - within tree susceptibility and whole tree susceptibility.

With regard to within tree susceptibility, it seems generally accepted that the susceptibility of sapwood to wood discoloration and decay increases with ageing of the wood (Good et al., 1955; Shigo, 1965). While no specific studies have been made on the relationship

between wood age and susceptibility or wood age and response ability, it seems probable that the increased susceptibility of older wood is related to the general decline in level of cellular properties which accompanies ageing in wood tissue. Observations on many cellular properties have shown that there is normally a progressive radial decrease in levels from the cambium to the pith or sapwood-heartwood boundary. Properties studied include moisture content (Stewart, 1967), carbohydrate resources (Priestley, 1962b), nitrogen content (Merrill and Cowling, 1966a), inorganic elements (Wardell and Hart, 1970b) and cytological properties (Frey-Wyssling and Bosshard, 1959).

There are limited reports of treatments altering whole tree susceptibility to wood rotting fungi. In most cases no attempt was made to relate the observed changes in susceptibility to physiological changes in the wood. These results mostly included external factors that act through the tree and were generally factors that reduced tree vigour.

In a brief review on factors altering susceptibility of stone fruits to S. purpureum, Grosclaude (1968) noted soil conditions were considered important. Generally, trees of low vigour growing on poor soils were more susceptible and showed less recovery than vigorous trees on good soils. Periods of root asphyxiation (Grosclaude, 1967) also increased susceptibility. It has been reported that the general decline in tree vigour brought about by such factors as overcropping, increased the susceptibility of apple trees to T. versicolor (Doepel, 1965). Mochizuki (1962) showed that the decline in tree vigour through overcropping was associated with decreased carbohydrate levels in the tree.

Baxter (1957a, 1957b) reported that potassium fertilisation increased tree vigour and lowered the incidence of decline and dieback in orchards.

The results of Wade (1968) are of particular interest. Phosphorus deficiency in young apple trees was found to markedly increase their susceptibility to T. versicolor. The effect of phosphorus deficiency contrasted with that of other low vigour inducing treatments (particularly nitrogen deficiency), which had no effect on susceptibility. No attempt was made to elucidate the physiological changes occurring in the trees, which led to the increased susceptibility, In field trees however, Darbyshire (1967) found no relationship between the incidence of dieback due to T. versicolor and the levels of phosphorus, potassium or calcium measured in current seasons shoots.

As well as these observations on factors altering whole tree susceptibility, the nutritional environment of the wood has also been proposed as being important in influencing the susceptibility of living wood to decay fungi. In vitro, experiments by many workers have shown that the addition of nitrogen (particularly organic nitrogen) normally increased the susceptibility of wood to decay, while the addition of carbohydrates decreased it (Findlay, 1934, 1941; Schmitz and Kaufert, 1936, 1938; Kaufert and Behr, 1942; Darbyshire, 1967; Darbyshire et al., 1969). Cowling and Merrill (1966) proposed that variations in the endogenous nitrogen content of wood could account for differences in decay resistance. With Populus grandidentata, wood of high nitrogen content from annual increments close to the cambium, was significantly more decay susceptible than wood of lower nitrogen content from deeper

within the stem (Merrill and Cowling, 1966b). Seasonal variations in nitrogen content also influenced decay resistance in a similar manner, wood of low nitrogen content being less susceptible to decay (Levi and Cowling, 1968). Comparisons of the in vitro decay susceptibility of root and stem wood of a number of coniferous species, showed root wood, which contained more nitrogen and carbohydrates, was consistently more susceptible to decay than stem wood (Platt et al., 1965). Thus inherent nutritional status of wood may significantly alter its decay susceptibility in vitro. Direct extrapolation to explain susceptibility to decay in vivo, as attempted by some workers, may not be possible however.

The results of Merrill and Cowling (1966b) cited above, showed in vitro, young wood was more decay susceptible than older wood. The opposite is true in the living tree where older wood is more susceptible to decay than younger wood. On the basis of results in vitro with non-living wood, Darbyshire (1967) and Darbyshire et al. (1969) proposed the hypothesis that Trametes versicolor wood rot of apple trees was a low sugar disease. It was suggested that more extensive decay occurred when host tissue sugar levels were low, and that the fungus had limited capacity to attack and destroy the wood when sugar levels were high. Likewise Beever (1970) proposed a relationship between nutrient levels of the xylem sap of fruit trees and their susceptibility to Stereum purpureum. Both hypotheses ignored the modifying effects of the discoloration reactions on the nutritional environment of the wood.

SECTION I

Host-pathogen relationship, host reaction to wounding and fungal invasion and the effect of host reaction on the susceptibility of sapwood to decay.

INTRODUCTION

Limited work has been undertaken on the host-pathogen relation-
ship of Trametes versicolor in apple. It was considered that a good
understanding of the host-pathogen relationship was essential in order
to comprehend and interpret results from studies on susceptibility.

In the following section an examination was made of the host-
pathogen relationship, and the nature of the host reaction (response)
to wounding and fungal invasion determined. Formation and properties of
the zones of discolored sapwood produced as a result of wounding and
fungal decay were studied. The effect of the changes that occurred in
the wood in advance of fungal penetration, on the susceptibility of
the wood to decay by T. versicolor, was assessed using in vitro decay
tests.

MATERIALS AND METHODS

(A) Naturally infected limb material.

During the investigation, approximately two hundred naturally infected limbs were collected from orchards in the Huon Valley in Tasmania for general studies. Infected limbs were collected from different apple varieties, trees of different ages, stages of infection and from trees growing on different soil types and under different cultural conditions.

Limbs for the study dealing with the relationship between external symptoms and internal decay were collected from orchards in the Geeveston, Castle Forbes Bay and Cygnet areas during the winter of 1969. They were all of the Cleopatra or Sturmer Pippen (STP) varieties. Work in this thesis was standardised on these varieties because they were two major Tasmanian apple varieties known to be susceptible to T. versicolor. Limbs selected were 7-12 cm. in diameter at their junction with the main stem, showed at least 60 cm. of papery bark and a minimum of 50 cm. of symptom free bark between the papery bark and main stem junction. Each limb selected was the only infected limb on a tree. Five limbs were selected from trees on nine properties.

Limbs for the determination of moisture, pH, mineral content, extractable materials and decay resistance of discolored wood were also selected according to the above criteria. They were obtained in winter 1970, from adjoining areas of 50-year-old STP and Cleopatra trees, growing on a well drained slope on a property at Castle Forbes Bay. Four limbs of each variety were selected. The limbs were cut adjacent

to the main stem and the cut ends sealed in plastic bags to prevent moisture loss. Limbs were returned to the laboratory and stored in a cool room at 4°C until they could be sampled (within 4-6 hours of cutting).

Wounds and artificial inoculations studied in this section were made on 5-year-old STP trees growing in sand culture at the University of Tasmania (details in Section III).

(B) Culturing and isolation techniques

Trametes versicolor strain D4 (original isolate from apple limb, B. Darbyshire, 1964) was used throughout the study. T. versicolor strain DFP 12017, an isolate from rotted wood, was obtained from C.S.I.R.O. Division of Forest Products, Melbourne. Stock cultures were maintained on a medium of dextrose (20.0 g.), peptone or asparagine (5.0 g.), KH_2PO_4 (1.0 g.), $MgSO_4.7H_2O$ (0.5 g.), agar (15.0 g.), yeast extract (2.0 g.) and distilled water (1000 ml.). Liquid medium was as above, but without agar.

Antibiotics were added according to Martin (1950), to help control bacterial growth when isolating from some specimens. Isolations from infected limbs were made from surfaces exposed by splitting limb sections. The exposed surfaces were lightly flamed and small pieces of the wood were excised with a sharp scalpel onto the agar medium. Plates were incubated at 25°C and examined as necessary.

(C) Sampling for moisture and pH determination, mineral analysis,
     extractable materials and decay tests.

Samples for pH and moisture determinations were taken as
systematically as the irregular nature of the apple limbs allowed. The
vertical axis of each limb was marked prior to its removal. A 100 cm.
bolt was cut, 50 cm. in either direction from the point of maximum
extent of external symptoms. The bolt was then carefully cut back
to include the zone from the deepest point of decay penetration to
where the entire limb was completely rotted. This remaining material
was then cut into 3 cm. wide discs, each of which was labelled, sealed
in a plastic bag and stored in a cool room until sampled. Every second
disc was used for moisture and pH sampling and the remaining discs
were used for isolations, material for extraction and decay tests.
Blocks for moisture and pH samples were taken from the discolored zone
and the adjacent 0.5 cm. of sapwood, at four equally spaced points
around each disc, commencing at the point equivalent to the upper limb
surface. A sharp chisel was used to excise the blocks. A single central
sample was taken from the white rot zone. Half of each block was used
for moisture determinations and the other halves were pooled to give a
composite sample for each disc of sapwood, discolored wood and white rot
for pH determinations. Samples for decay testing were taken in a
similar manner from the remaining discs. The remaining material from
these latter discs was separated into discolored tissue and adjacent
sapwood, and a composite sample of the two tissue types was compiled
for each variety from the four limbs of each variety, for examination
of extractable materials.

The uneven nature of apple wood, due to limb traces, pruning cuts etc., made this sampling pattern difficult to maintain. Sometimes only two or even one sample could be obtained from a disc. This led to smaller sample numbers and lack of consistent positional samples in each tissue zone and it became necessary to average the results on a per limb basis.

(D) Moisture and pH determinations and mineral analyses.

1. Moisture was determined on an oven dry basis after drying the blocks at $105^{\circ}C$ for 48 hours.

2. pH was determined using indicators and glass electrode methods. The composite sample from each disc was cut into fine shavings (1-2 g.) with a scalpel, 15 ml. of distilled water added and the pH of the water extract determined after 4 hours with a Radiometer pH Meter 22. Little change in pH was observed even when the extraction period was extended to 24 hours. Methyl red, chlorophenol red and phenol red permitted measurement over a pH range from 4.20-8.40. The indicators were applied to the freshly cut surfaces of blocks of tissue.

3. Mineral analyses on the discolored and adjacent sapwood were carried out by the Tasmanian Government Analysts Department (details in Section III).

(E) Decay tests.

Blocks for decay tests were dried at 65°C for 48 hours and weighed. Decay tests were conducted using the method of Darbyshire (1967) and Darbyshire et al. (1969), except that 2% water agar was used as a supporting medium. The wood blocks were sterilised by auto-claving in a closed dry jar for 15 minutes. The blocks were then placed aseptically on the agar surface and allowed to equilibrate for 3 days. Jars were inoculated by placing two 0.5 cm. square mycelial pads of T. versicolor (from the outer margin of a 5-7 day old culture) on either side of the block. After incoulation, the jar lids were loosened a quarter turn and the jars incubated at 25°C for approximately 24 weeks. Relative humidity in the incubation room varied in the range 40-60%. After incubation the blocks were freed of mycelium, dried at 65°C for 48 hours and the percentage weight loss determined.

Two experiments were conducted. The first was preliminary and blocks of discolored wood were obtained from the discolored zones around infections in Cleopatra and STP limbs as described in (C). The blocks were irregular in size and shape, the number of blocks available for decay testing depending on the limb.

In the second experiment a large STP limb infected with T. versicolor was selected (winter 1970). A fairly large but not abnormal amount of discolored tissue extended below the maximum depth of fungal penetration. The extent of T. versicolor infection was checked microscopically and the discolored wood free of T. versicolor was separated from the rest of the limb. It was possible to cut

sufficient blocks of a small size (1.2-1.5 g.) from the discolored wood and the adjacent sapwood, to conduct a 2x2 factorial experiment with two strains of T. versicolor with 9 replications. The wood used in this experiment was judged to be between 30-50 years old for the discolored wood and 20-40 years old for the sapwood controls. Two strains of T. versicolor were used to compare the wood decaying ability of an isolate from a living tree (D4), and an isolate from rotted wood (DFP 12017).

(F) Method of wounding and artificial inoculation.

The major requirement of this study was a method which was relatively quick, effective and adapted to large numbers of inoculations. Spore suspensions and dowel inoculations were rejected, as spore suspensions involved problems of growing, collecting and maintaining the viability of spores of a known strain, while the small size of limbs to be inoculated, precluded the use of dowels. The method of Darbyshire (1967) and Wade (1968) was therefore adopted. Branches were cut back to the wood of the required age and branch stubs inoculated with discs of mycelium on agar, cut from the outer edge of a 5-7 day old culture of T. versicolor with a sterile cork borer, of similar diameter to the stub to be inoculated. The inoculated stubs were covered with aluminium foil for one month to prevent drying out. In this Section all wounds and inoculations were made on wood less than 5-years-old.

(G) Fixation and sectioning.

Material was fixed in formalin-acetic-alcohol (Jensen, 1962).
For long term storage, tissue was removed from FAA after fixing and
stored in 70% alcohol.

A Leitz Base Sledge Microtime No.1300 was used for sectioning.
Sections were cut directly from small blocks of fixed or fresh
material, normally at a thickness of 20-30µ

(H) Histochemical techniques.

Fungal hyphae were detected using Cartwright's (1929) method.
Hyphae stain blue and lignified tissue red by this method. In tissue
that contained living cells care was required in interpretation. The
picro-aniline blue stained nuclei and cytoplasm blue and in addition
aggregated the cytoplasm in such a manner that it resembled fungal
hyphae. Fresh and fixed tissue stained equally satisfactorily.

Starch was detected using aqueous iodine-potassium iodide
solution (Jensen, 1962) and lipids by Sudan black B (Jensen, 1962).
Sections were mounted in glycerol.

An 0.5% solution of 2,3,5-triphenyl tetrazolium chloride (TTC)
was used to detect living cells. The dehydrogenase enzymes present in
living cells reduce TTC to an insoluble red formazan. Thin sections do
not react, and small blocks of tissue were incubated in the solution
in darkness for 4-36 hours at 25°C (depending on tissue age),
sectioned and mounted in glycerol.

Polyphenols were detected by the method of Gagnon (1967).
It is based on the Hoepfner or Hoepfner-Vorsatz reaction in which poly-
phenols react with nitrite and acetic acid to produce a yellow color,
which is converted to a red color with excess alkali. Reeve (1959b)
believed the method to be specific for catechol tannins. Gagnon's
method gave a color ranging from pink to cherry red with polyphenols in
apple tissue. Acidic 0.5% $FeCl_3$ was also tried for the detection of
tannins (Jensen, 1962).

Wound gum was stained by a variety of reagents. Orcinol
(3,5 dehydroxy-toluene) reacts with hexoses, pentoses and hexuronic
acids (Jensen, 1962). With wound gum it gives a purple coloration.
The method was modified from that of Rawlins and Takahashi (1952).
Fresh or fixed sections were placed in   4% orcinol in 95% alcohol, for
5-10 minutes. Sections were then placed on slides and a few drops of
concentrated HCl added. Color developed in a few minutes, after
which the sections were mounted in glycerol. Schiff's reagent stained
gum deep purple. The oxidation of polysaccharides by periodic acid
produces aldehydes which react with leucofuchsin to produce intense,
stable colors. The procedure of Jensen (1962) was used. Gum was also
stained pink by ruthenium red and red by phloroglucinol in 18% HCl
(Jensen, 1962). The staining of gum with the Maule reaction for
lignin (Rawlins and Takahashi, 1952) and the hydroxylamine-ferric
chloride test (Jensen, 1962) were tried. The specificity of this
latter reaction for pectins depends on the conversion of pectin methyl
esters to pectin hydroxamic acid by alkaline hydroxylamine. The
acids are converted to red complexes with ferric irons.

Polyphenoloxidase and peroxidase were detected using α-napthol (Shain, private communication). Sections (30μ) were incubated for 12 hours at $37^{\circ}C$ in $3.5 \times 10^{-4}M$ α-napthol in 0.1M phosphate buffer pH 5.6. The α-napthol was dissolved in absolute alcohol before the addition of buffer and the concentration of ethanol in the reaction mixture was kept constant at 1%. Controls used included reaction mixtures minus substrate, wood sections steamed prior to reaction and the addition of 0.1M sodium azide to the reaction mixture. For peroxidase the above procedure was followed but 0.15% H_2O_2 was added to the full reaction mixture. Checks without H_2O_2 were included.

(I) <u>Assay for total phenolics and extraction procedures</u>.

1. Total phenolics were assayed using the Folin-Denis reagent following the procedure of Swain and Hillis (1959). The reaction depends on the oxidation of the phenolic compounds by phosphomolybdate to give colored derivatives. Tannic acid was used as a standard.

2. <u>Extraction procedures</u>.

The efficiency of hot and cold extraction methods in removing material from discolored and normal apple sapwood were compared.

(a) Hot extractions

The method of Da Costa and Rudman (1958) was used. The wood was ground in a Wiley Mill No.1, dried at $65^{\circ}C$ for 24 hours and then extracted in a Soxhlet apparatus with (i) ether (10 ml. per g. sawdust) for 2 hours to remove oil, fats and waxes, (ii) absolute methanol (10 ml. per g. sawdust) for 16 hours to remove the phenolics,

and (iii) refluxed for 2 hours with 0.1N NaOH (20 ml. per g. sawdust) to remove alkali extractable material. Extracts were concentrated in a rotary film evaporator at 40°C and stored in a small volume of the extracting solvent at 4°C. A known aliquot of each extract was dried at 65°C for 48 hours to determine the total dry weight of material extracted, and a suitable aliquot of each extract was assayed for phenolics.

(b) Cold extraction.

A modification of the method of Somers and Harrison (1967) was used. The dried sawdust was extracted at room temperature in a closed container, on an end-over-end shaker with (i) two changes of absolute methanol (10 ml. per g. sawdust, 4 hours per change), and (ii) two changes at 50% aqueous methanol (10 ml. per g. sawdust, 4 hours per change). The extracts were combined and aliquots taken for dry weight determinations and phenolic assays. Total phenolics were pre-cipitated from the extracts using lead acetate as described by Somers and Harrison (1967).

(J) <u>Fractionation and chromatography of phenolics</u>.

The recovered phenolics were fractionated on Sephadex G-25 Fine following the method of Somers and Harrison (1967). A 50 x 1.5 cm. column was used with 50% aqueous acetone as the eluting solvent. Each elution curve was determined using a Hitachi-Perkin Elmer UV-Vis Spectrophotometer in 1 cm. cells at 450 mμ.

Preliminary thin layer chromatography was carried out on 20 cm. square plates of Whatman CC41 microgranular cellulose (250μ thick) layered in 50% ethanol. A 10 cm. solvent run was used with 6% acetic acid and butanol-acetic acid-water (64:10:27) at room temperature.

OBSERVATIONS AND RESULTS

(A) General effects and external symptoms of attack by Trametes versicolor in apple trees.

The general effects and symptoms of the disease have been described and illustrated by Thomas and Raphael (1933), Birmingham (1936), Anon., (1956), Ward (1957), Doepel (1962), Darbyshire (1967). T. versicolor is a wound parasite, which attacks the woody parts of a tree. Once established it can bring about the death of individually affected limbs and over a period, the destruction of the entire tree (Plate 1). While the term 'dieback' is commonly used to describe the disease, the fungus can readily penetrate upwards into limbs and grafts causing the typical white rot and death of the upper parts of the limbs. Death of the upper portions of limbs so affected, usually results from the eventual severance of the transpiration stream.

The most obvious external symptom associated with the disease was that known as 'papery bark' (Plates 2,3). The papery bark consisted of the phellem, which separated and lifted along the phellogen. The separation and peeling of the phellem was due to unusual activity

PLATE 1   A tree in an advanced stage of attack
          by T. versicolor. Note the dead limbs
          and papery bark.

PLATE 2   Showing the boundary between papery bark and healthy
           bark. On the upper portion of the limb the bark is
           deeply cracked and dried. The lower portion shows
           the production of papery bark - callus outgrowths
           associated with cracking of the bark and peeling of
           the phellem.

PLATE 3   As in Plate 2 but after the limb was stored in
           a cool room for several months. Note further
           development of callus outgrowths, general
           unevenness of the bark due to meristematic
           activity within the bark, and peeling of the
           phellem.

of the vascular cambium or meristematic activity of phloem or phelloderm parenchyma cells. This activity resulted in the production of varying amounts of undifferentiated callus cells beneath or within the bark, causing cracking and unevenness of the bark surface (Plates 2,3). Generally these symptoms were confined to the border region between healthy and affected bark, but in some cases larger areas of bark showed this activity. Later the bark cracked more deeply and dried, leading to the appearance illustrated in the upper portions of Plates 2 and 3. Often a marked wet and slimy condition of the bark surface occurred between the initial disturbances and the eventual deep cracking and drying. Doepel (1962) observed a similar condition. The development of papery bark in artificial inoculations in young apple wood followed the same type of pattern as that seen in natural infections (Plate 4).

It should be emphasised that the papery bark symptom was not entirely restricted to Trametes versicolor infections. Several cases were investigated where it was not possible to isolate T. versicolor or other wood-rotting Basidiomycetes from limbs, although the classical papery bark symptoms were present and actively extending. Further evidence that this symptom was not specifically associated with T. versicolor infection, comes from the work of Howard and Banwell (1969), who found that maiden Cox trees stored at 36°F (2·2°C) from February to June (Northern Hemisphere) with quantities of apple fruit, showed callus eruption from wounds, bark splitting and a papery bark condition.

Observations indicated that there was a marked seasonal pattern in the longitudinal extension of the papery bark. Greatest

PLATE 4   An artificial inoculation of T. versicolor
           in 2-year-old STP wood. Note typical papery
           bark symptom.

development was seen in late autumn and winter, both in natural and artificial inoculations. Activity of the type illustrated in Plates 2 and 3 was minimal or ceased altogether during the late spring and summer, the margin between healthy and affected bark becoming cracked and dried. Measurements made on artificial inoculations verified these observations, but these results will be considered in detail in Section III.

Cool humid conditions seemed to favour the type of meristematic activity described, and the subsequent production of papery bark. Circumstantial evidence for this was obtained when a number of bolts of infected limbs, taking in the region between healthy and infected wood, were stored in a cool room at $4^{o}C$ from mid-August 1970 until November 1970. The development and extension of the bark symptoms continued under these conditions (compare Plates 2,3). The results of Howard and Banwell (1969) confirm that this type of activity can occur at low temperatures, although in their case a definite stimulus (apple fruit) was necessary for it to occur.

(B) Internal features of T. versicolor attack in apple limbs and the general response of sapwood to wounding.

    1. Natural infections

Longitudinal and transverse sectioning of limbs infected with T. versicolor revealed a consistent relationship between wood tissue and fungal pathogen. In terms of gross morphology several zones were always discernible around infections. The designation of the zones was based on macro- and microscopic examination and was modified from the

system used by Good et al. (1968). Plates 5 and 6 and Figure 1 illustrate the pattern.

(a) Central white rot zone

Fungal degradation of the wood had proceeded to its greatest extent in this zone. The texture of this zone was soft and punky and was usually fairly uniform throughout the zone. The wood fabric showed the typical features of a white rot decay-enlargement of pits, formation of bore holes, thinning of cell walls and loss of color.

(b) Zone of incipient decay

This zone was intermediate between the white rot zone and the outer discolored zone of sound wood. Breakdown of xylem and ray parenchyma cell contents, extraneous material in the vessels and the commencement of actual wood decay, occurred in this zone.

(c) Discolored or stain zone

Surrounding the zones of white rot and incipient decay was a zone of discolored but sound tissue. Hyphae penetrated into the inner discolored zone but little or no breakdown of cell contents had occurred. The lateral extent of this zone was seldom more than 0.5 cm. and often less. The ray and xylem parenchyma cells of this zone were dead and their lumens filled with irregular masses of brown material.

(d) Sapwood-discolored wood transition zone

This zone was usually not differentiated by color although it sometimes showed as a water soaked shadow around the discolored zone (Plate 5). The cells in this zone showed characteristic changes and in active infections were eventually transformed into discolored tissue.

PLATE 5   Transverse sections of Democrat limb infected with
           T. versicolor. Note central white rot zone, zone
           of incipient decay, outer discolored zone and the
           sapwood-discolored wood transition zone, which
           shows as a dark shadow around the discolored zone.

PLATE 6   Longitudinal sections of Cleopatra limb (left)
and Democrat limb (right) infected with
T. versicolor, showing the various zones.

<u>FIGURE 1</u>

Diagramatic illustration of the zones differentiated
around <u>Trametes</u> <u>versicolor</u> infections in apple sapwood.

WHITE ROT
ZONE

INCIPIENT
DECAY ZONE

DISCOLORED
WOOD

SAPWOOD-
DISCOLORED
WOOD TRANSITION
ZONE

BARK

SAPWOOD

PITH
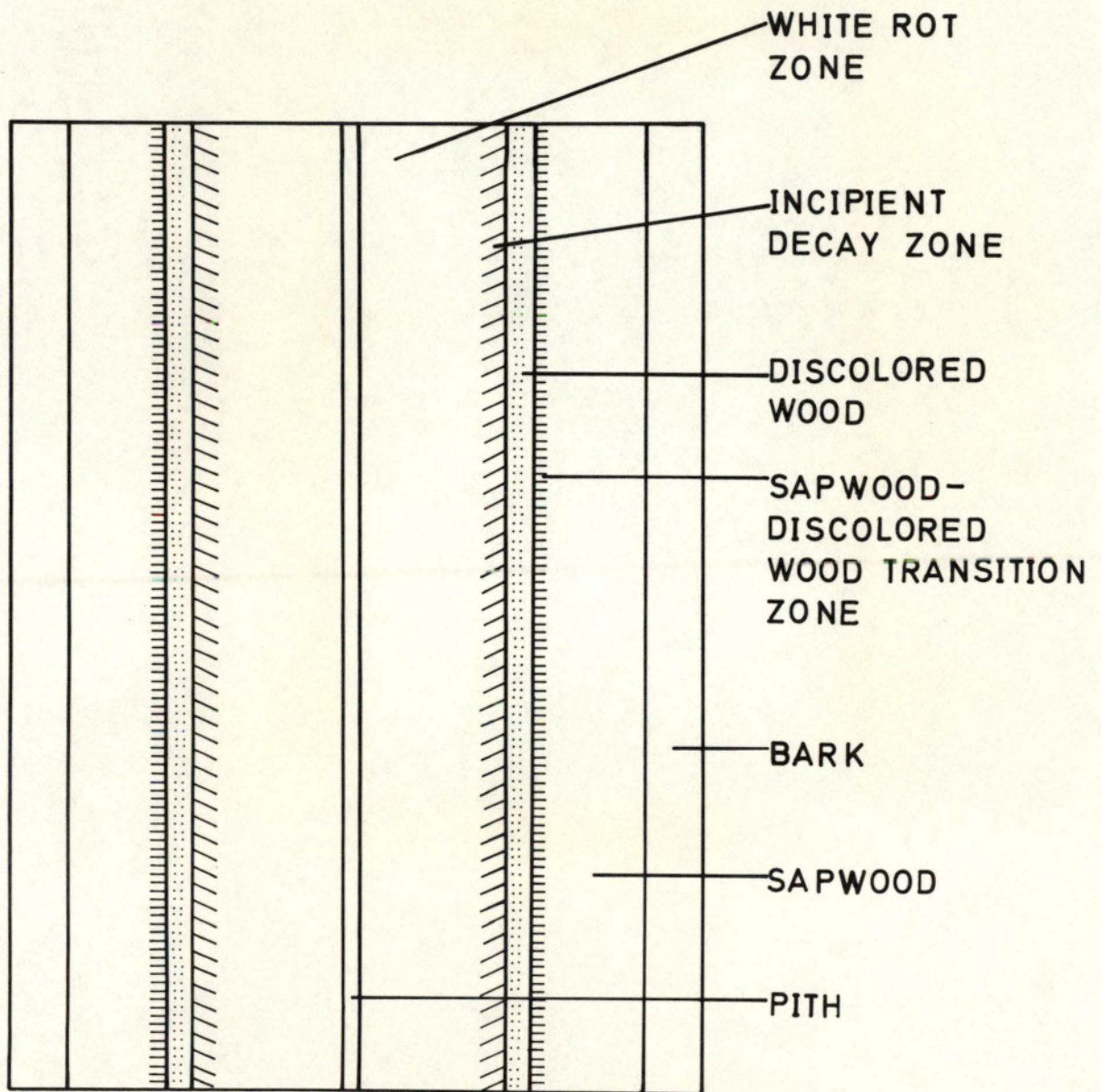
The transition zone seldom extended more than a few millimetres laterally. The discolored zone and the sapwood transition zone were surrounded by normal sapwood, except where the limb had been completely decayed.

The distribution of the fungus in diseased limbs was determined by the incubation of discs from infected limbs in humidity chambers, isolation of the fungus from the wood and direct microscopic examination of thin longitudinal sections. It had also been hoped to use fluorescent antibody staining for the localisation and the positive identification of the pathogen in the wood but as preliminary work was unpromising, this approach was abandoned.

Incubation of discs resulted in a dense outgrowth of fungal mycelium from the outer edge of the white rot zone and the zone of incipient decay after a few days. Growth from the central white rot zone was slower and did not always occur. Cartwright's stain showed hyphae were never present in the wood outside the discolored zone, although they often penetrated its inner margin and sometimes almost to its outer margin. The vessels of the outer white rot and incipient decay zones were frequently filled with wefts of fungal hyphae. A large number of isolations confirmed that the fungus was always confined within the zone of discolored wood escept where the entire limb had been penetrated by the fungus.

The most frequent infection state of T. versicolor was where the fungus penetrated down a limb towards the base of a tree. The infection front, as marked by the discolored zone, showed a V-shape in one dimension or a conical shape in three dimensions (Plate 7).

PLATE 7   Typical V-shaped infection front in two
STP limbs infected with T. versicolor.
Deepest penetration occurs in the oldest
xylem. The upper portions of the limbs
have been completely decayed.

Greatest penetration occurred in the centre (oldest) part of the limb. In active infections therefore, the discolored zone, which represented the boundary between affected and healthy tissue, showed lateral movement towards the vascular cambium and longitudinal movement towards the base of the limb. Distal of the point where the infection front reached the vascular cambium around the limb circumference, the wood was completely white rotted (Plate 7). The distance from the point where the infection front reached the cambium to its greatest depth in the limb centre, varied from a few centimetres to a metre or more. The extent of discoloration at the lateral margins of infections was normally quite restricted (Plates 5, 6). The extent of discoloration below the maximum point of fungal penetration in the centre of a limb varied from the normal few centimetres up to half a metre.

Entry of the pathogen on the upper portions of limbs therefore, resulted in a downward penetration of the limb by the fungus behind a conical boundary of discolored tissue. Variations of this pattern occurred frequently enough to warrant noting.

Variations from the normal pattern.

(a) ~~An extreme development of the normal situat~~ion

A number of infected limbs were examined where a narrow central core of white rot and accompanying discoloration extended down the centre of the limbs for up to 1-1.5 metres, usually in the absence of external symptoms. In other cases, long columns of discoloration without decay were found.

(b) Infection of wounds not allowing immediate entry to the central xylem

Infection of surface wounds on limbs usually resulted in a

localised area of decay and discolored tissue just under the wound
(Plate 8). No doubt such infections could eventually cause decay of the
whole limb.

(c) Infection of wounds on limb bases or main stems, resulting
in upward penetration of limbs by the fungus

If the pathogen gained entry low down on limbs it often grew
downwards and outwards until it eventually killed the entire ~~diameter~~
~~of~~ sapwood. The limb section distal to the rot died and became hard,
brown and dry, without decay. The bark dried and shrank onto the wood
and did not show papery bark symptoms.

In other cases of this type, particularly when the pathogen
entered a limb from the main stem, it penetrated up the limb, leaving
sufficient living sapwood and phloem to maintain transpiration and
translocation until extensive rotting of the limb had occurred. An
apparent feature of this type of infection was that the advance dis-
colored zone was usually very extensive.

2. Artificial inoculations in young apple wood

Experimental inoculations were always made on branch stubs
(less than 5-years-old), in a manner to ensure infection across the
entire exposed area of the xylem. The resulting infections showed the
same form and type of zonation as in natural infections in larger
limbs (Plate 9). The white rot and incipient white rot zones were
surrounded by a dark discolored zone. Lighter discoloration (sapwood-
discolored wood transition zone) extended from the zone of maximum
discoloration until the normal sapwood color was attained (Plate 9).

PLATE 8   Infection of a surface wound on a STP limb,
          by T. versicolor. The cambium was disrupted
          and the limb has become asymmetrical in shape
          with later growth.

PLATE 9   Artificial inoculations with T. versicolor in
           3-year-old STP limbs. Note zonation of the
           infected wood. The lighter discolored sapwood
           extending below the zone of maximum dark dis-
           coloration represents the sapwood-discolored
           wood transition zone.
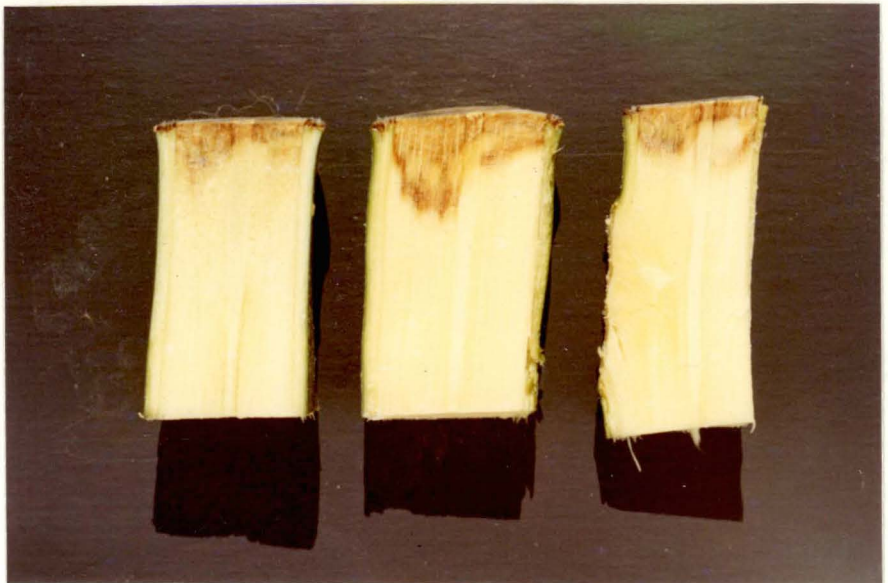
PLATE 10   Discoloration present under non-sterile but un-
           inoculated wounds on 3-year-old STP limbs six
           months after wounding. Discoloration under such
           wounds was often mottled in appearance.

The distribution of external symptoms was associated with disturbances in the underlying wood.

3. Sterile and non-sterile wounds in apple wood.

Both sterile and non-sterile wounds in apple sapwood resulted in a brown to dark brown discoloration of the underlying wood. The extent and shape of the discolored zone depended on the type of wound, its age and sterility.

Discoloration below cut stubs took the form of a cone with the most extensive discoloration towards the centre of the stub (Plate 10). In terms of gross appearance, the only difference between sterile and non-sterile wounds was that in the latter, discoloration was darker and more extensive.

(C) Relationship between external symptoms and internal decay in natural infections.

From observation it appeared that the disturbance of the bark previously described, was normally associated with changes in the underlying wood. Papery bark usually did not extend more than a few centimetres past the point where visible discoloration could be seen in the underlying wood. However, in a number of infections examined, papery bark extended much further longitudinally (either all around the limb or on one side of it) than any discoloration in the wood. The reason for this is unknown.

Discoloration and decay in the centre of limbs usually extended further than the external symptoms. Of forty-five limbs

infected with T. versicolor (single infected limb per tree) examined in winter 1969, internal decay and discoloration extended farther than external bark symptoms in forty-two cases (Table I). In three cases the situation was reversed by a few centimetres. In the majority of limbs examined, the farthest extent of decay and discoloration lay in the range 10-40 cm. past the last external symptoms.

(D) Fungal penetration in relation to xylem structure.

One of the outstanding features of both natural and artificial infections was the conical shape of the infection front. Wade (1968) stated that in young wood, the pith served as the primary pathway of longitudinal penetration for the fungus. The relationship between xylem structure and fungal penetration was therefore considered in some detail in artificial inoculations.

A series of artificial inoculations were made on 3-year-old wood of healthy 5-year-old STP trees in winter 1969. Five inoculations were cut and examined every month for eleven months. The extent of hyphal penetration was measured microscopically in longitudinal sections at 0.5 mm. intervals across the diameter of the xylem. The results for the five inoculations were averaged for each sampling time. Figure 2 shows the mean results at every second sampling time while Figure 3 shows the mean penetration over ten sampling times as related to xylem structure.

From Figures 2 and 3 it can be seen that the greatest hyphal penetration occurred in the inner secondary (2°) xylem and in the oldest wood of the 2° xylem (3-years-old at time of inoculation,

TABLE I

Relation between external symptoms and internal decay and discoloration in limbs infected with T. versicolor.

Distance internal decay and discoloration extended past bark symptoms. Range (cm)	Number of limbs within range.
10-0	3
0-10	6
10-20	14
20-30	11
30-40	8
> 40	3
Total number of limbs	45

changing to 4-years-old over the period of sampling). As the wood decreased in age, the extent of hyphal penetration declined towards the vascular cambium where hyphal penetration was a minimum. The penetration in the pith (part of the 3-4-year-old wood) was less than that in the 3-4-year-old 2^O xylem. Pith penetration was greater than that which occurred in the outer half of the 2^O xylem.

The results of this experiment indicated that the major path of longitudinal penetration for the fungus was in the vessels of the secondary xylem and not in the pith column. The results also illustrated the fact that the greatest fungal penetration and decay normally occurred in the oldest xylem. Examination of a large number of natural infections and artificial inoculations over the course of this study, showed the former result was not invariable. In some cases maximum hyphal penetration occurred in the pith, but it was concluded that in the majority of cases the main path of penetration of limbs was in the vessels of the xylem (generally the oldest 2^O xylem), followed by a more restricted radial spread into the rays, xylem parenchyma, vessels and fibres.

Three other points of interest arose from the results. Firstly, the pathogen did not appear to be restricted in penetrating from one annual ring to another. The fungus apparently penetrated laterally into the new wood elements as they were formed without difficulty, although penetration in the youngest tissue was not very great. Secondly, the results indicated that the fungus was active throughout the year, and did not seem to show any marked variation in its activity within the wood with season. This was in contrast to the pattern shown for the

FIGURE 2

Length of hyphal penetration in 3-4-year-old Sturmer Pippen
sapwood (inoculated July 30, 1969), measured one month after
inoculation and at two monthly intervals thereafter. (Each
plot represents the mean values of five inoculations.)
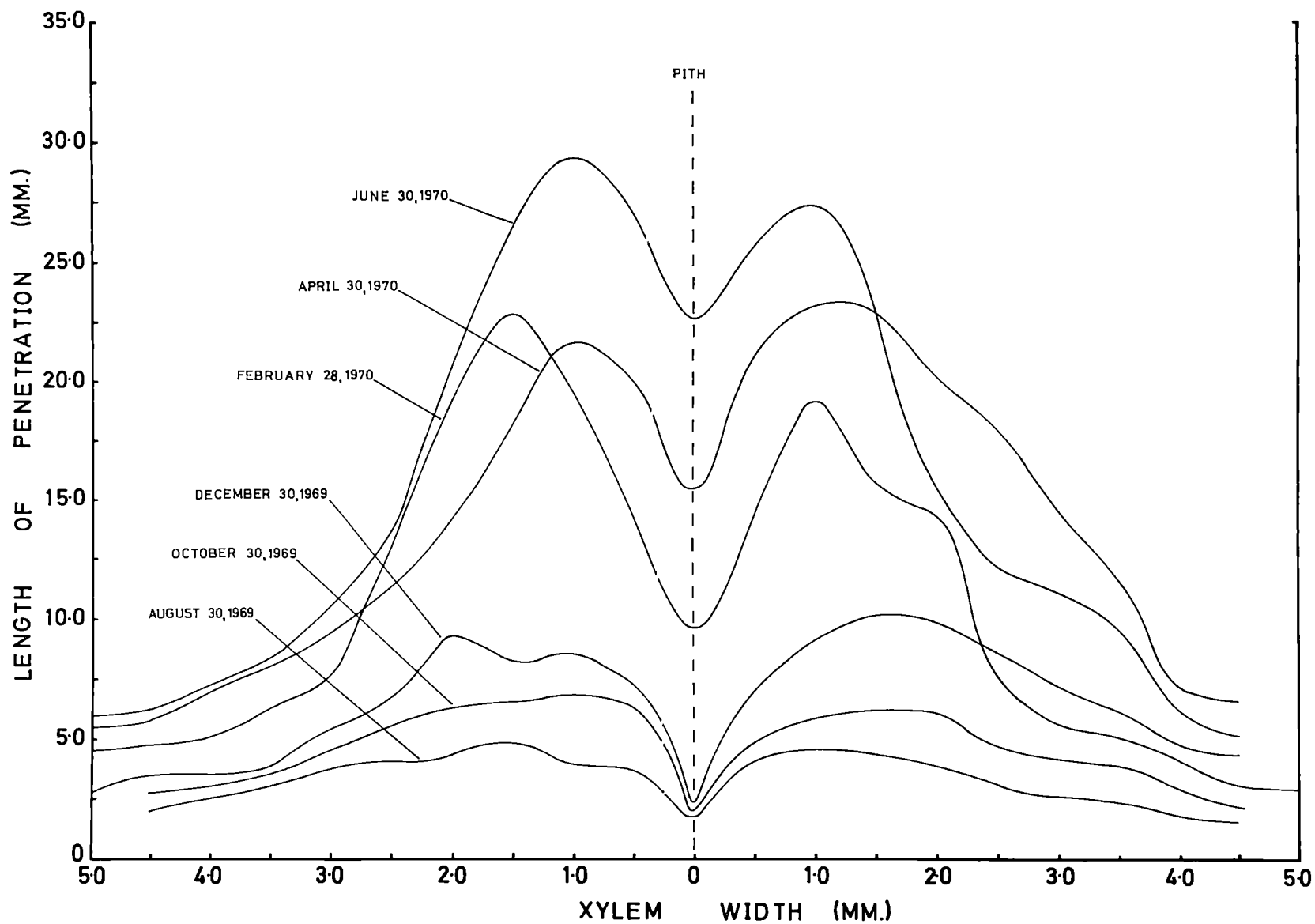
FIGURE 3

Penetration of 3-4-year-old Sturmer Pippen sapwood over an eleven month period in relation to xylem structure. The length of penetration is the mean from ten sampling times.
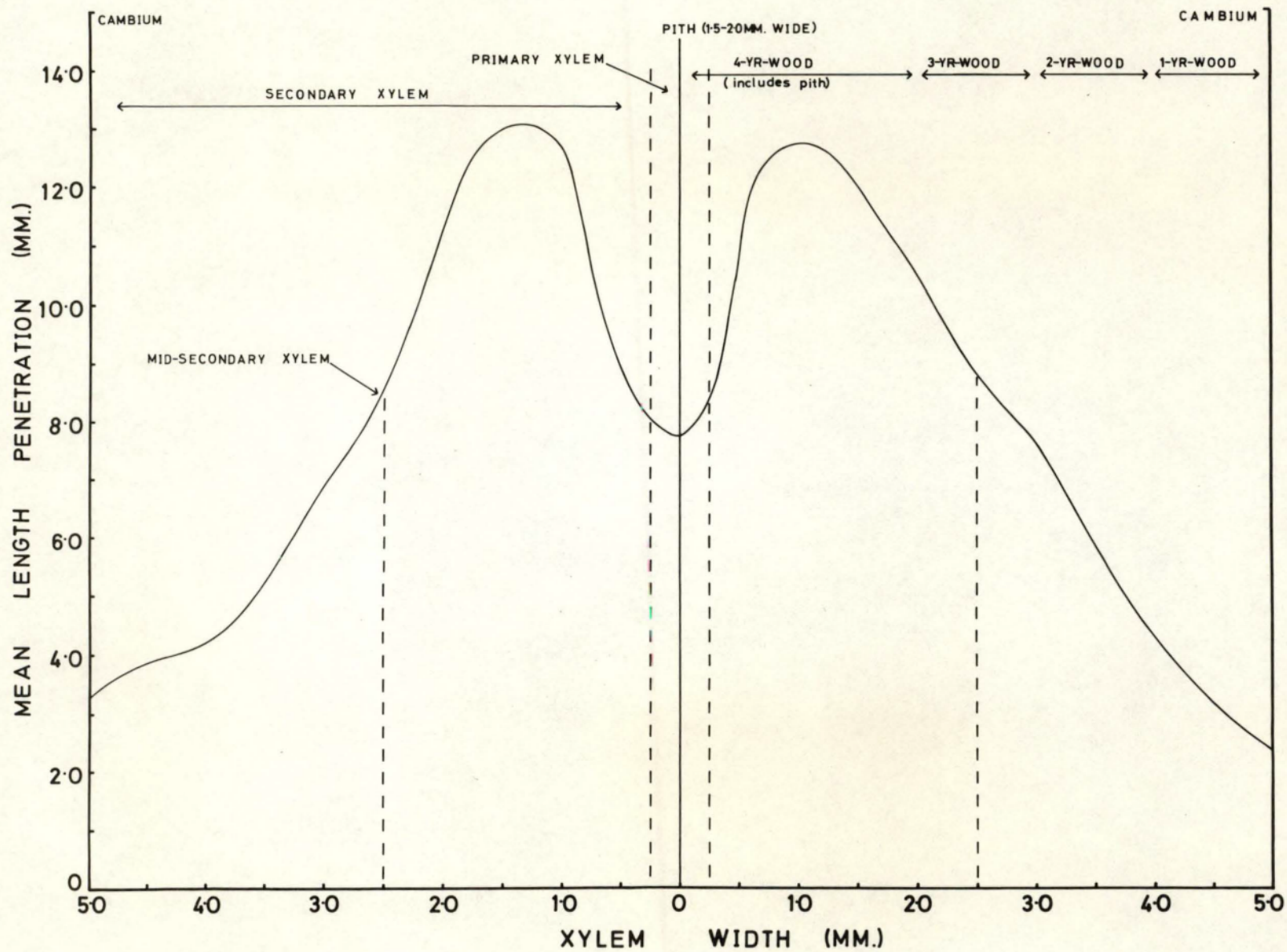
the development of papery bark, which in this experiment showed a seasonal occurrence as described earlier. Thirdly, the results in Figure 2 illustrated the typically slow rate of penetration by T. versicolor observed in young apple limbs (approximately 2.6 mm. per month in the inner xylem of these limbs). The rate of penetration was higher in the older wood than in the younger wood.

(E) Formation and properties of the zones of discoloration associated with T. versicolor infections and wounding of apple sapwood.

The distinct discolorations of sapwood observed around fungal infections and wounds indicated drastic changes occurred in the physiology of the wood. It was decided therefore, to examine some of the properties of these zones, and identify the major changes that occurred in their formation.

1. Color.

Plates 5-10 inclusive, illustrate the type of coloration encountered on freshly cut wood surfaces in natural and artificial infections and under wounds. The dark brown of the discolored zone was gradually transformed by fungal activity to the bleached color of the central white rot zone. Outwards, the brown of the discolored zone changed to the normal creamy-yellow color of the sapwood. On the lateral margins of natural infections the change from discolored wood to sapwood color was usually abrupt, but at the apex of the infection the transition zone was more extensive.

In natural infections the discolored zone was seldom more than a dark brown in color, while under artificial inoculations almost black stains were sometimes encountered. Discoloration was due to the colored deposits present in the xylem and ray parenchyma cells and vessels and discoloration of the cell walls. Intense discolorations were associated with heavier coloration of the wound gum and parenchyma cell contents than normally observed. Discoloration under wounds was not always uniform and often showed a mottled appearance (Plate 10).

2. Presence of reserve materials

Starch was entirely absent from the white rot, incipient decay and discolored zones around fungal infections, and the discolored areas around wounds. In natural infections, starch decreased in quantity as the discolored zone was approached from the sapwood, until the ray and xylem parenchyma cells adjacent to the discolored zone contained no starch. Plate 11 illustrates the transition.

In artificial inoculations and under wounds, the decrease in starch levels in the parenchyma and ray cells coincided with the formation of wound gum in the adjacent vessels. Complete disappearance of starch always occurred as the dark discolored zone was approached. In healthy sapwood, ray and xylem parenchyma cells contained some starch all the year round. The amount varied considerably from an autumn-winter maximum to a minimum in spring and early summer.

Carbohydrate analyses of a small number of discolored wood samples from around natural infections in large limbs showed very low levels of soluble sugars and starch (usually <2 mg./g. dry wood),

low enough to suggest contamination of the sample by small amounts of sapwood during sampling. Hemicelluloses appeared to remain at approximately the same level in discolored wood as in normal sapwood (about 240-270 mg./g. dry wood). Analyses of the adjacent sapwood (taking in the sapwood-discolored wood transition zone) showed that the disappearance of starch was compensated for by a rise in the levels of soluble sugars, and there was no evidence that the net carbohydrate content of that zone was significantly altered.

3. Presence of living cells.

2,3,5-triphenyl tetrazolium chloride (TTC) gave good differentiation between dead and living cells. Living cells were absent from white rot, incipient decay and discolored zones in all sections examined. Many of the fungal hyphae in the outer white rot zone and incipient decay zones stained heavily with TTC, indicating their viability.

Around infections and wounds, the loss of starch from ray and xylem parenchyma cells as the discolored zone was approached, was paralleled by the loss of ability of these cells to reduce TTC (Plate 12). On the lateral margins of infections, cells immediately adjacent to the zone of discolored cells showed no staining, but the amount of precipitate increased to a normal level over a range of 20-30 ray cells.

Red staining globules of material were observed in cells adjacent to the discolored zone (Plate 13). Hart (1963) observed a similar phenomenon in other species but gave no indication of their nature. These globules appear to be lipid as the red formazan
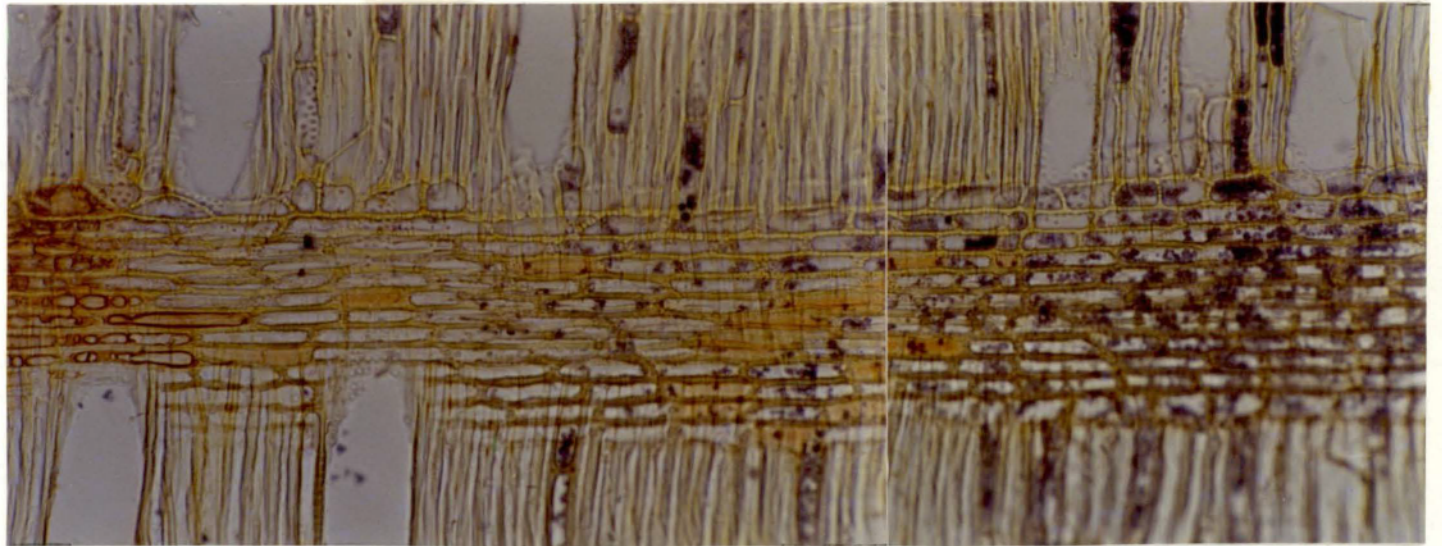
PLATE 11   Decrease in the starch content of cells of the sapwood-discolored
wood transition zone as the zone of discolored cells (left) is
approached laterally along a medullary ray. The light brown ray
cells in the centre of the Plate are apparently already dead and
stain intensely for polyphenols. (Natural infection STP limb.
I-KI stain.   x 200)

PLATE 12   Decrease in intensity of staining with TTC of cells in the
            sapwood-discolored wood transition zone as the discolored
            zone (left) is approached laterally along a medullary ray.
            Note red staining globules in cells adjacent to discolored
            zone. (Natural infection STP limb.   x 200)
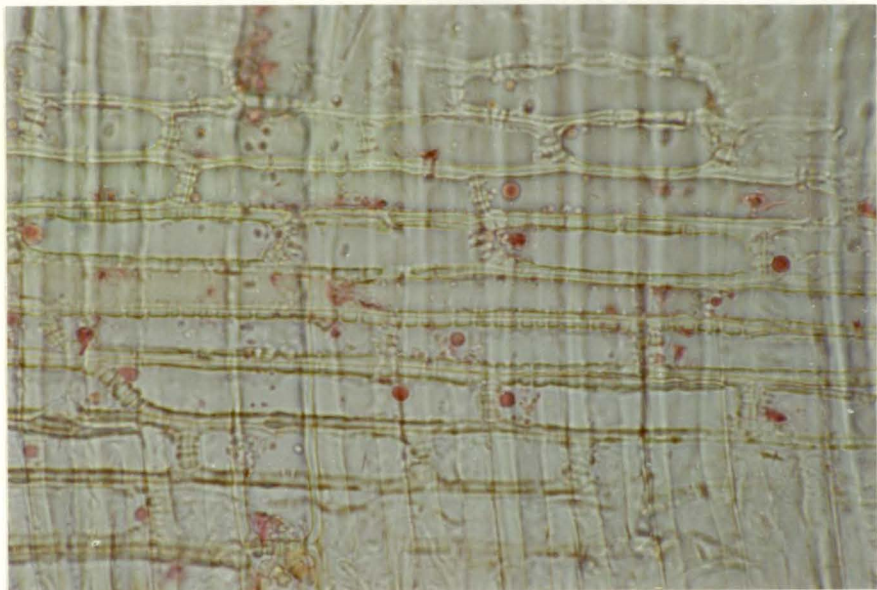
PLATE 13   TTC staining globules in cells adjacent to the
            discolored zone. There is often more than one
            globule per cell suggesting that they are not
            nuclei. (x 500)

PLATE 14   Globules in ray cells adjacent to the dis-
            colored zone stained with Sudan black B.
            (x 500)

precipitate is very soluble in lipid (Conn, 1961) and the globules
stain black with Sudan black B (Plate 14).

Although not investigated in detail, nuclear staining properties
altered markedly across the sapwood-discolored wood transition zone.
The picro-aniline blue element of Cartwright's stain readily stained the
nuclei and cytoplasm of normal cells but no nuclei were seen in the cells
adjacent to the discolored zone. The nuclei decreased in size and
became misshapen before finally disappearing.

4. Presence of extraneous material in the xylem and ray parenchyma
    cells in discolored tissue.

The ray and xylem parenchyma cell contents were transformed at
the edge of the discolored zone into a brown material which was
encrusted around the lumen walls, filled the pit cavities or occurred as
irregular masses or globules within the lumen (Plates 11, 15, 16). As
noted, the material in the parenchyma cells accounted for most of the
color of the discolored zones. Usually, some light discoloration of the
cell walls in these areas was noticeable. Under artificial inoculations
and wounds in young wood, a more intense light yellow-brown discoloration
of the vessel, fibre and parenchyma walls and contents sometimes
occurred (Plate 17). This discoloration was seen in the tissues adjacent
to the discolored zone and formed a halo around it. The discoloration
was most intense in the outer xylem and stained orange with the
Hoepfner-Vorsatz reaction (Plate 17). Why this discoloration did not
occur consistently is not known.

PLATE 15   Typical appearance of ray and xylem
            parenchyma cells in the discolored zone
            under a T. versicolor infection in a 3-
            year-old STP limb. Note the irregular
            morphology of the material in the xylem
            and ray parenchyma cells and the wound
            gum in the vessels. (x 200)

PLATE 16   Magnification of centre portion of Plate 15 showing
           more clearly the appearance of the brown material in
           the cells. Note the empty appearance of the cells
           prior to their transformation to the discolored
           state, the brown material in the pit cavities and,
           in comparison with Plate 11, the greater quantity of
           brown material in the discolored cells of young wood
           than in older tissue. (x 500)

PLATE 17 Discoloration of cell walls and contents some-
times observed adjacent to the zone of
discolored cells (right), in young apple wood.
Note the occasional red staining ray and xylem
parenchyma cells in the orange staining zone.
(Hoepfner-Vorsatz staining.   x 32)

The ray and xylem parenchyma cell contents were degraded by the fungus. This occurred in the zone of incipient decay and the process was notable by the manner in which the cell contents tended to round off and be gradually eroded away (Plate 18).

The exact nature of the material in the ray and xylem parenchyma cells is unknown. The strong staining of ray and xylem parenchyma cells for polyphenols immediately prior to their transformation to the discolored state, suggests the material could be oxidised phenolics.

Normal ray and xylem parenchyma stained a light pink-orange with the Hoepfner-Vorsatz reaction, although some stained more intensely. As the discolored zone was approached, the intensity of staining in most cells decreased and only a portion of what appeared as debris in cell lumen reacted (Plates 19, 20). Sometimes isolated cells in the transition zone stained a bright red (Plate 19) but this appeared abnormal. A consideration of Plates 11 and 19 indicated that the cells which stained bright red in the transition zone were partially discolored and almost certainly dead. At the edge of the discolored zone, all cells developed a strong polyphenol staining reaction. Once the cells had discolored staining of the brown contents was variable. Cell contents often only darkened with the reaction and no red coloration could be seen. On other occasions (Plate 20), positive red staining was observed in discolored cells. The evidence therefore indicated the discolored material was at least partially phenolic in nature.

The material in the cells was chemically inert. Prolonged extraction with hot methanol and ethanol failed to remove it. Acid hydrolysis (N H_2SO_4) for one hour removed only a part of it but boiling

PLATE 18 Breakdown of the ray and xylem cell contents
and wound gum in the zone of incipient decay,
by T. versicolor. Blue material in the vessels
is hyphal and the reddish-purple staining
material in the vessels is the remains of
wound gum deposits. (Cartwright's stain.
x 200)
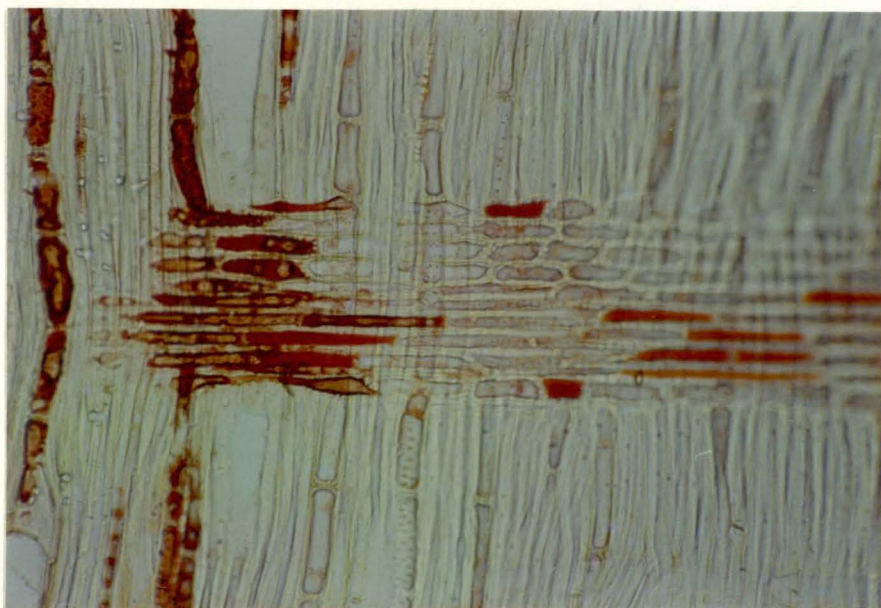
PLATE 19   Polyphenol staining in cells in the sapwood-
discolored wood transition zone. Note in most cells
only a portion of the contents stain a light orange-
pink prior to the transition to the discolored state,
but some apparently already dead, stain a bright red.
All cells seem to pass through this stage of red
staining prior to the complete transformation of the
cell contents. (x 200)
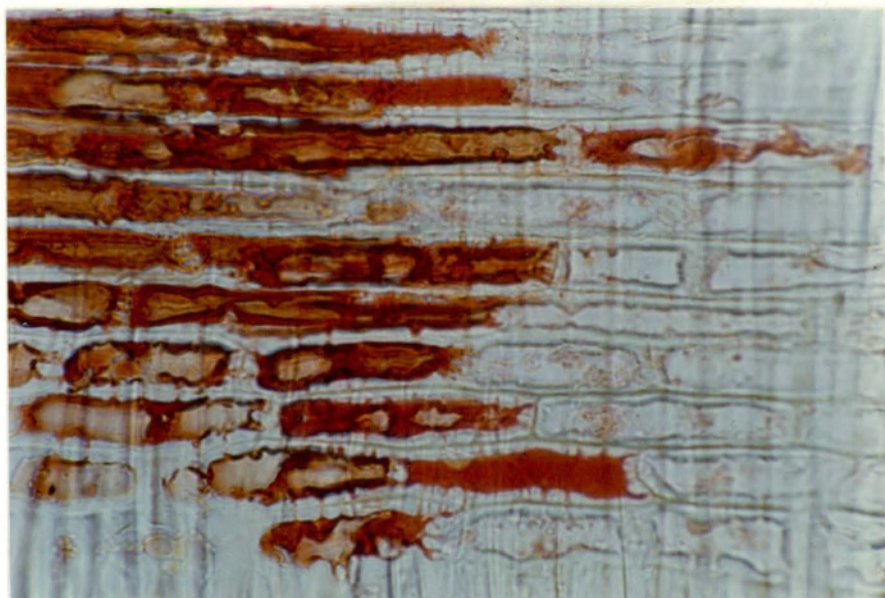
PLATE 20   Higher magnification of ray cells at the sapwood-
           discolored wood transition, stained for polyphenols. Note
           appearance of cells in the pre-transition state (right)
           and the post-transition state (left) and the red
           staining developed in cells during the transformation of
           the cell contents. (x 500)

thin sections in 5N NaOH for half an hour removed most of the cell contents.

Beneath the surface of uninoculated wounds, another type of transformation of cell contents was commonly observed in pith, ray and xylem parenchyma. The transformation involved the apparent death of the cells, and the degeneration of the starch grains within the cells, into brown irregular deposits or wall encrusting masses (Plate 21). The starch grains appeared to aggregate, with gradual loss of staining ability with iodine-potassium iodide solution. The material formed from the starch was possibly amylodextrin but the final product looked no different from that previously described in discolored cells. It did not stain with the Hoepfner-Vorsatz reaction and could be degraded by T. versicolor. The in situ transformation of starch was normally restricted to irregular zones of cells adjacent to wound surfaces and below this zone discoloration of cells occurred in the usual manner. On rare occasions in situ starch degradation occurred beneath fungal infections in young wood, when starch grains sometimes appeared to be trapped in cells at the edge of the discolored zone.

5. The presence of wound gum, its distribution and properties.

Tyloses are not formed in apple wood. Tyloses form when the pit aperture from ray cells to vessel is greater than 8-10μ (Chattaway, 1949). The pit aperture in apple wood falls below this limit. Young apple wood (arbitrarily defined as wood less than 5-years-old) formed large amounts of material in the vessels which will be referred to as gum or wound gum.

PLATE 21   The disorganisation and aggregation of starch
           granules within ray and xylem parenchyma cells.
           It was often observed beneath wound surfaces.
           (I-KI stain.   x 500)

In the discolored zones and adjacent sapwood transition zone around infections or wounds in old wood, the vessels remained empty of gum apart from occasional small plugs or globules (Plate 22). Only when the infection or wound traversed younger wood was any appreciable gum formation observed.

Around artificial inoculations and wounds in young wood, copious quantities of gum were formed in the vessels. Gum formation resulted in a barrier around the zone of fungal activity or under a wound, with actual physical blockage of the vessels across the entire diameter of the xylem and often to an extensive depth in the wood (Plate 23). The pattern of gum formation in the areas around wounds or infections was superimposed on the other transitions in cellular organisation that were observed. The distribution of gum and its relation to changes in other cellular properties was considered in detail for the case of an established infection in young wood.

In the incipient decay zone most of the gum had been destroyed by fungal activity. The fungal hyphae grew through and around the gum masses, gradually dissolving them (Plate 18). A close association between the fungal hyphae and the gum seemed necessary for its dissolution. The most complete blockage of the vessels by gum was in the discolored zone and in the sapwood transition zone. In the discolored zone the gum was discolored yellow to brown and cracked and brittle in appearance. Over the sapwood-discolored wood transition zone, the amount of starch in the ray and xylem parenchyma cells increased to its normal sapwood level. The opposite trend was observed with the amount of gum in the vessels, which decreased from a maximum in the

PLATE 22   Illustrating the restricted gum formation in the discolored
zone surrounding a T. versicolor infection in approximately
30-year-old STP wood. Note the decayed wood to the left of
the discolored zone and healthy sapwood to the right.
(Orcinol.   x 32)

PLATE 23   Portion of the gum zone below an infection of
           T. versicolor in a 3-year-old STP limb. Note
           the extent of gum formation and the normal
           appearance of the ray and xylem parenchyma
           cells in the gum zone. (Cartwright's stain.
           x 32)

discolored and adjacent sapwood to zero in the normal sapwood. Thus starch containing xylem parenchyma and ray cells were frequently adjacent to vessels in which large amounts of gum were present (Plate 24). The greater the distance from the discolored zone the less color possessed by the gum, the newest formed gum being almost colorless. The tissues that contained the majority of gum were therefore, outside the zone of maximum discoloration. These tissues were themselves discolored to some extent and often had a brown water-soaked appearance (Plate 9).

Gum formation was a property of the sapwood-discolored wood transition zone in young wood. The formation of gum occurred concurrently with the disappearance of starch, a decline in TTC reducing ability and the loss of nuclei from xylem and ray parenchyma cells. Gum originated from ray and xylem cells as observed by Chattaway (1949) and Talboys (1968). It appeared therefore, that gum synthesis was a reaction of living cells but that gum formation did not result in immediate death of the cells. The changes in cellular properties and pattern of gum distribution under an infection in young wood are summarised in Figure 4. A similar pattern of changes occurred under wounds in young wood.

Gum was mainly confined to the vessels although occasionally it was seen in the fibres (Plate 25). The occurrence of gum in the fibres does not appear to have been reported previously. Gum never occurred within the lumens of the ray and xylem parenchyma cells.

The morphology and density of gum deposits in the vessels varied considerably. It could be present as dense homogenous plugs filling vessel lumens over a long distance (Plate 23), but frequently it was most irregular in form and distribution within an individual vessel member.

PLATE 24   Starch containing ray and xylem parenchyma
            cells deep within a zone of gum formation.
            (I-KI stain.   x 200)

FIGURE 4

Diagramatic representation of the normal pattern of gum distribution
and other cellular changes observed in advance of decay by _Trametes
versicolor_ in young apple sapwood (less than 5-years-old).

Key: I   White rot zone.

      II   Zone of incipient decay.

            Fungal degradation of wound gum, xylem and ray cell
            contents and the commencement of wood decay occurred
            in this zone. Grades into III.

      III   Zone of maximum discoloration.

            Xylem and ray parenchyma cells dead and filled with
            irregular brown deposits. Cell walls possibly discolored.
            Vessels filled with discolored wound gum which appeared
            cracked and brittle. Grades into IV.

      IV   Sapwood-discolored wood transition zone.

            Adjacent to discolored zone,ray and xylem parenchyma
            appeared empty. Cells did not contain nuclei, starch or
            reduce TTC (except the lipids) but some stained for poly-
            phenols. Copious amounts of light colored wound gum in
            the vessels. Passing towards normal sapwood starch and
            TTC reducing ability of cells increased and staining for
            polyphenols decreased. Wound gum in vessels decreased in
            amount and color.

      V   Normal sapwood.

            No gum in vessels, plentiful starch and high TTC reducing
            ability in ray and xylem parenchyma.

      VI   Pith.

            No advance host reaction involving cell death. Hyphal
            penetration of cells resulted in their death.

PAPERY
BARK

BARK

SAPWOOD

PITH

I

II

III

IV

V

VI
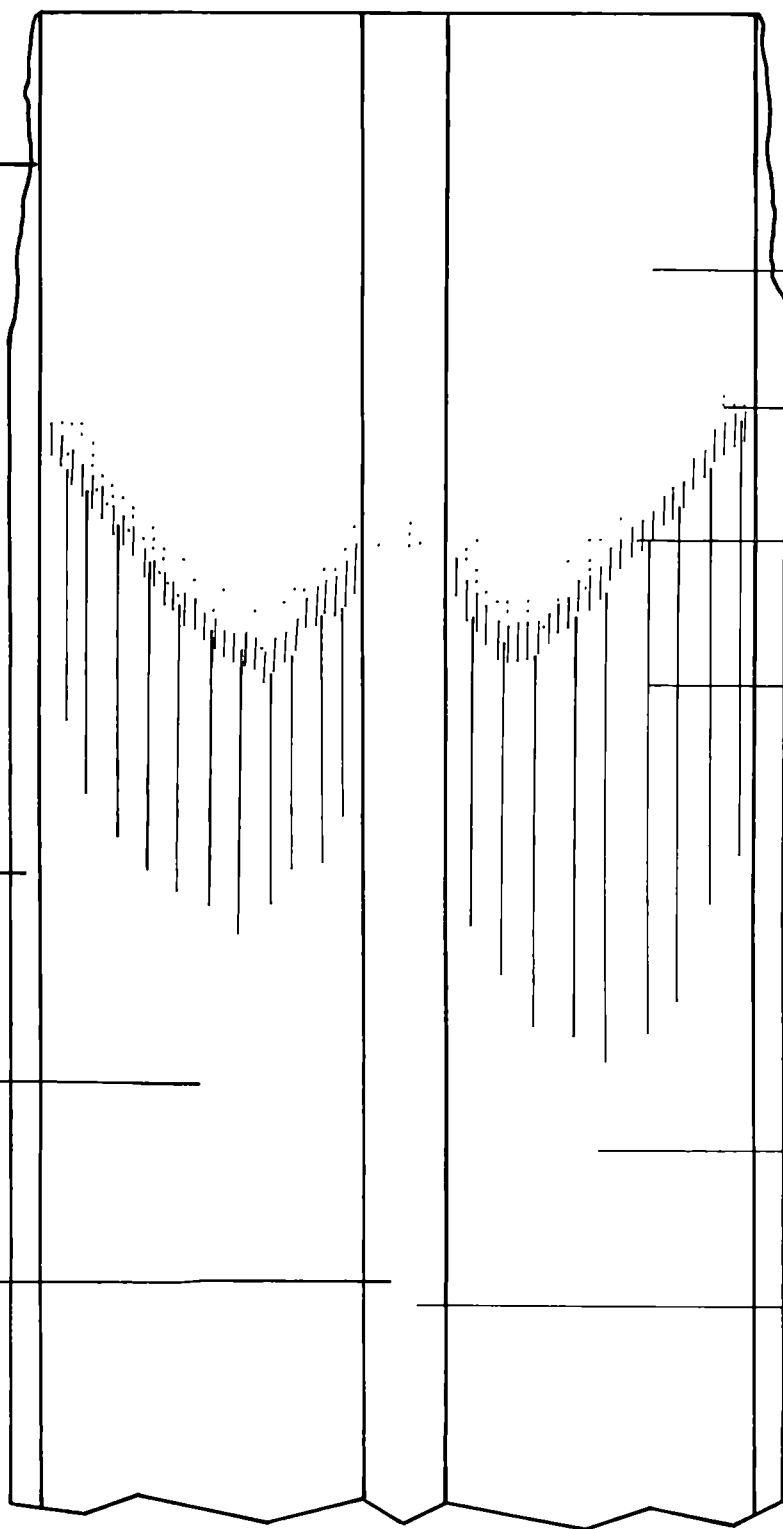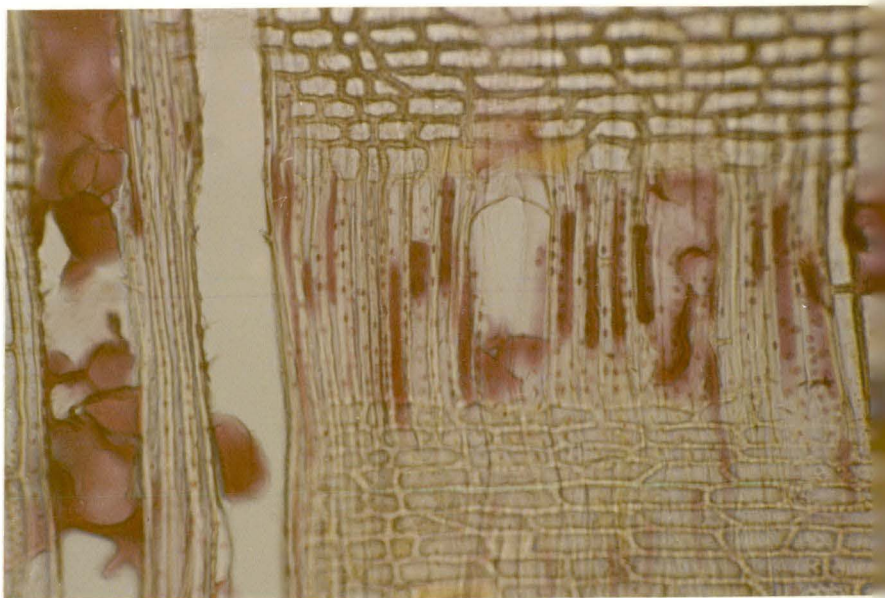
PLATE 25   Wound gum in vessels and fibres.   (Orcinol. x 200)

Gum globules which appeared similar to the tyloses of other tree species, were observed. Chattaway (1949) also observed gum deposits simulating tyloses. Commonly gum deposits of varied morphology were present in the one vessel. Plates 23-27 show a variety of forms of gum deposits. The intensity of staining of gum with various reagents also varied considerably.

It appeared that there was more than one type of wound gum, although this was not investigated in detail as there seemed little difference in their staining properties. The more common type was dense and homogenous in appearance and usually present as plugs, globules or irregular masses of different form. The other type observed was granular in appearance and usually present as amorphous masses (Plate 26). Talboys (1968) reported two types of wound gum in plums.

The effectiveness of gum to act as a physical barrier and seal off the underlying tissue from the external atmosphere in wounds and inoculations in young wood, was illustrated using a pressure chamber (described in Section III). Ten cm. lengths of wounded or inoculated branch stub were sealed in the pressure chamber with their freshly cut ends immersed in 1% acid fuchsin in the chamber and the inoculated or wounded stub end protruding. The pressure required to force dye through the control lengths of sapwood was very low - about 10 p.s.i. It was almost impossible however, to force dye through tissue that had been inoculated or wounded for more than a few weeks. Sustained pressures of up to 400 p.s.i. for several hours could not force dye through the wound gum zone in most cases, illustrating the effectiveness of this material in sealing the tissue and re-establishing a closed system.
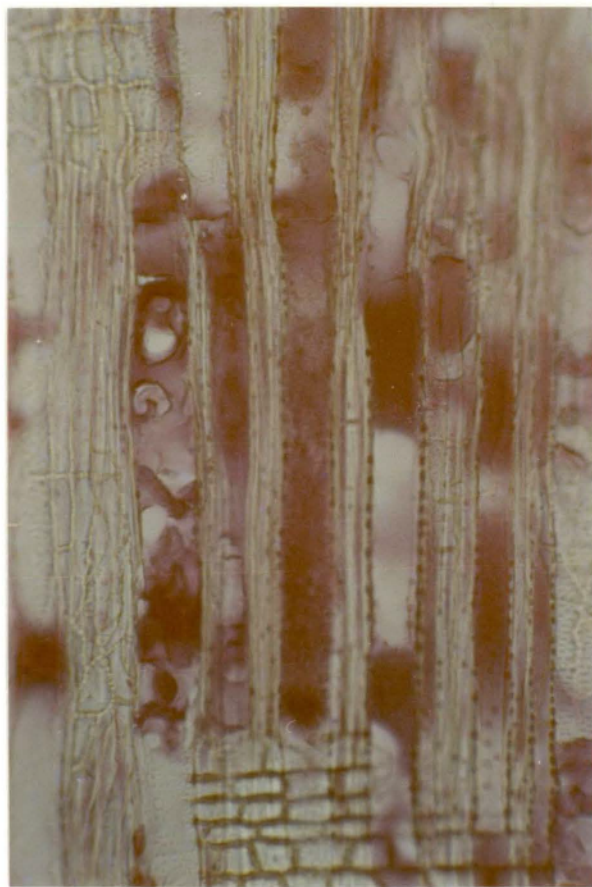
PLATE 26   The two types of gum observed in apple wood.
           Note the smooth homogeneous type (left) and
           the grainy type (centre). (Orcinol. x 200)

The results agreed with those of Swarbrick (1926) concerning the effect-iveness of blocking. No attempt was made to examine the rapidity of blocking with season, with or without inoculations etc.

Apple wound gum had similar properties to those described for wound gum of other hardwood species (Literature Review). The freshest gum was colorless but with ageing it darkened. The extent of discoloration was seldom more than a dark yellow-brown although dark olive-black gum deposits were observed. Generally, the most discolored gum was that which the fungus was degrading. The gum hardened and cracked with ageing and became less reactive to staining and other chemical agents.

Gum was insoluble in hot and cold water. Even prolonged boiling of thin sections failed to solubilise gum or reduce the intensity of its staining with orcinol. Gum was insoluble in organic solvents including methanol, ethanol, ether, acetone and chloroform and cold concentrated acids such as hydrochloric and nitric acids. Boiling of thin sections (30μ) in 5N NaOH for half an hour, resulted in a gelatinisation of the gum and a loss of its ability to stain with orcinol.

A number of histochemical reactions give some indication of the chemical composition of wound gum. Orcinol (specific for hexoses, pentoses and hexuronic acids) reacted with gum to produce a deep purple coloration (Plate 25, 26). The intensity of staining made the gum readily distinguishable from the surrounding tissues and was an excellent means of assessing the distribution of gum. The concentrated HCl used to develop the color, hydrolysed the starch in living cells and cells that were not discolored appeared clear and empty. The reaction indicated that

the gum was polysaccharide in nature. This was confirmed by the staining of wound gum purple by Schiff's Reagent. This reagent offered a means of studying the distribution of starch and gum with a single stain and permanent preparation.

Cartwright's stain served as a useful agent for studying the distribution of gum especially in relation to fungal hyphae. The newest gum often stained a purple color with Cartwright's stain but after a short period of maturation it stained red (Plate 27).

Phloroglucinol in 18% HCl stained gum cherry red. The Maule reaction for lignin did not stain wound gum. Staining with phloroglucinol indicated the presence of aromatic aldehydes rather than lignification (Rawlins and Takahashi, 1952).

The pectin stain, ruthenium red, was taken up weakly by gum but the intensity of staining was low compared with orcinol or phloroglucinol. Sterling (1970) showed staining with ruthenium red to be stereospecific and dependent on negative charges spaced $4.2\overset{\circ}{A}$ apart, with sufficient space to accommodate the ruthenium complex. Pectin has an abundance of such sites and the result indicated wound gum either had such groupings or contained pectic material. The fact that wound gum did not stain with the hydroxylamine-ferric chloride reaction, claimed to be specific for pectins (Reeve, 1959a), was evidence against wound gum containing pectic material.

The presence of polyphenolic material in gum was checked with acidic $FeCl_3$ and the Hoepfner-Vorsatz reaction. $FeCl_3$ gave no staining of polyphenols in gum or in any other tissue. The Hoepfner-Vorsatz method stained older gum an orange-brown, while the newer gum was often
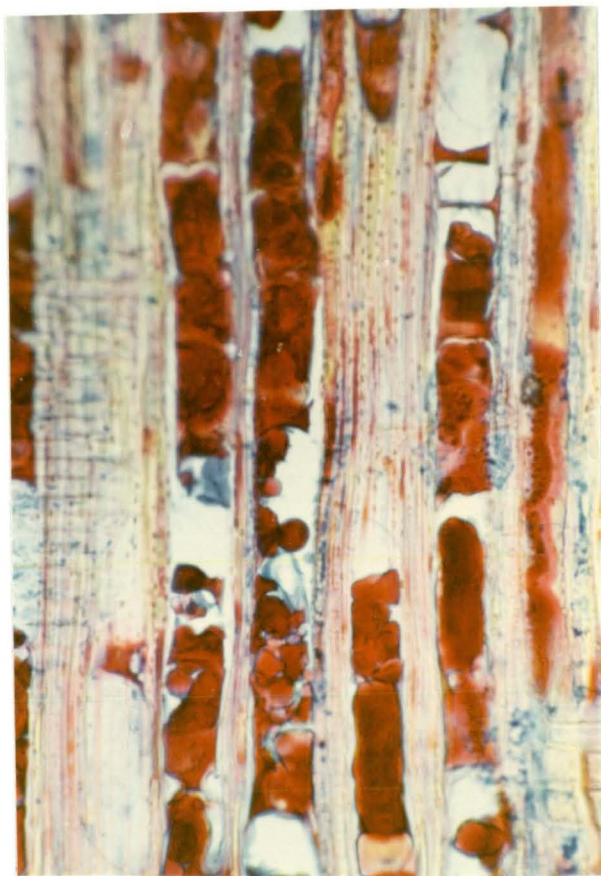
PLATE 27   Staining of gum with Cartwright's stain.
            Note the varied morphology of the gum
            deposits. (x 200)

colored a light orange-pink. While the test was not strongly positive it suggested the presence of polyphenolic material in the gum.

Using α-naphthol as a substrate it was possible to show that freshly formed gum contained both polyphenol oxidase and peroxidase. The youngest gum took on a weak purple coloration after incubation with α-naphthol which indicated the presence of these enzymes.

6. Moisture content of white rot, discolored zones and adjacent sapwood.

Difficulties encountered in sampling prevented statistical analysis of the results. The figures for moisture content obtained from the sampling of four limbs of two varieties are presented as means for the different zones in Table 2. While the results were somewhat variable general trends were evident.

Relative to the discolored tissue and sapwood, the white rot zone showed m marked drop in moisture content. The rotting process resulted in the loss of water and presumably the decay process would cease altogether if the moisture content dropped sufficiently.

The difference between the discolored zone and adjacent sapwood, at least in winter, did not appear to be very great. The overall mean moisture content of the discolored zone was higher than that in the sapwood but this was not so within all individual limbs sampled. The moisture content of sapwood varies with season, (Stewart, 1967) and it is possible that the difference between discolored and sapwood tissue could be greater at other times of the year. In addition, on freshly cut limb surfaces, the sapwood adjacent to the discolored wood sometimes

TABLE 2

Mean moisture contents of zones around infections of $\underline{T}$. versicolor
in Cleopatra and Sturmer Pippen limbs.

(Limbs sampled July–August 1970, moisture contents expressed
as % oven dry basis at 105°C.)

Variety and limb no.		Central white rot zone	Discolored zone	Adjacent sapwood
Sturmer Pippen (STP)	1	81.7 (5)[*]	107.2 (15)	104.6 (14)
	2	111.1 (3)	102.1 (17)	110.5 (18)
	3	64.2 (5)	114.3 (15)	102.9 (13)
	4	74.0 (6)	109.0 (15)	104.0 (15)
	Mean	82.8 (19)	108.2 (62)	105.6 (60)
Cleopatra	1	70.6 (15)	104.4 (21)	86.9 (18)
	2	75.8 (7)	105.5 (26)	108.7 (21)
	3	78.6 (8)	102.5 (26)	87.0 (26)
	4	69.9 (6)	104.4 (20)	95.4 (20)
	Mean	74.0 (36)	104.2 (97)	94.5 (85)

[*] Figures in brackets represent number of samples in each mean.

developed a dry flinty appearance after drying for a short time, suggesting a lower moisture content in that zone.

The results agreed in general with those of Good et al. (1955, 1968) who found discolored tissue in maple decayed by Fomes igniarius to have a higher moisture content than adjacent sapwood, with a big drop in moisture level in the white rot zone.

7. pH of white rot, discolored zone and adjacent sapwood.

The mean pH values for each zone in the limbs sampled are presented in Table 3.

The results from glass electrode studies indicated that the pH of discolored wood was similar or slightly less than that in the sapwood. In the Sturmer Pippen the pH of the discolored zone tended to fall. This may not have been a real effect however, as the lateral extent of the discolored zone was not very great in some infections, and it could not be guaranteed that wood free from fungal influence was sampled.

The pH in the white rot zone dropped to the range 5.1-5.7, the figures being more variable than those obtained in the sapwood and dis-colored zones. In other samples of advanced decay, pH's in the range 4.5-5.0 were recorded.

Tests with a number of indicators on fresh wood surfaces gave general agreement with the glass electrode results. It was difficult to assess indicator colors in the discolored tissues.

Additional evidence that the reactions involved in discoloration of the sapwood led to a slight decline in pH came from the incubation of fresh sterile wood at high humidity. The pH was observed to drop from an initial figure of 6.1 to 5.8-5.9 after 20 days.

·A rise in pH has been reported for discolored tissue around wounds and fungal decays in many hardwoods (Good et al., 1955; Hart, 1965, 1968). In apple however, it appeared the pH of discolored tissue tended to decline compared with sapwood.

8. Mineral content of discolored wood and adjacent sapwood.

Preliminary analyses of the contents of various mineral elements in the discolored and adjacent sapwood were conducted on composite samples of discolored wood and sapwood, from the four limbs of each variety previously used for moisture and pH measurements (Table 4).

While the results were limited they indicated that potassium, calcium and magnesium tended to increase in the discolored wood while the nitrogen content decreased compared with the adjacent sapwood. The only other element to show any marked variation in level was sodium, although the results were not consistent. The trace elements showed very little difference between the wood types, while phosphorus was notable for its extemely low levels in these samples.

The results generally agreed with those of Good et al. (1955), Hart (1968) and Shain (1971) who found K, Ca and Mg levels increased in discolored sapwood of a range of hardwoods and softwoods. Good et al. (1955) believed the increased pH in discolored sugar maple to be related to the increased mineral content of the discolored wood compared with sapwood. The differences in level of K, Ca and Mg between sapwood and discolored wood recorded by Good et al. (1955), Hart (1968) and Shain (1971) were much greater in magnitude than those found in apple and could account for the rise in pH observed in discolored wood of the species they studied.

TABLE 3

Mean pH levels of zones around infections of <u>T. versicolor</u>
in Cleopatra and Sturmer Pippen limbs.
(Limbs sampled July–August 1970, 4 hour cold water extract of shavings.)

Variety and limb no.		Central white rot zone	Outer discolored zone	Adjacent sapwood
Sturmer Pippen	1	5.6 (6)*	6.0 (6)	6.2 (6)
	2	5.6 (5)	6.0 (8)	6.3 (8)
	3	5.2 (4)	5.8 (8)	6.0 (8)
	4	5.5 (3)	5.8 (7)	6.2 (7)
	Mean	5.5 (18)	5.9 (29)	6.2 (29)
Cleopatra	1	5.5 (5)	6.0 (7)	6.1 (7)
	2	5.1 (7)	6.0 (7)	6.0 (7)
	3	5.6 (8)	6.2 (8)	6.0 (8)
	4	5.7 (6)	5.8 (6)	6.0 (6)
	Mean	5.5 (26)	6.0 (28)	6.0 (28)

* Figures in brackets represent the number of samples in each mean.

TABLE 4

Mineral analyses of the discolored wood and adjacent sapwood from around $\underline{T}$. versicolor infections in Cleopatra and Sturmer Pippen limbs.

Wood type	Variety	Parts per million (dry weight)											
		K	Ca	N	Mg	P	Na	Mn	Cu	Fe	Al	Zn	B
Sapwood	Cleopatra	1400	1500	1060	300	2	112	5	2	24	9	2	4
	Sturmer Pippen	1000	1500	1040	280	4	30	3	2.4	28	9	3	1
	Mean	1200	1500	1050	290	3	71	4	2.2	26	9	2.5	2.5
Discolored wood	Cleopatra	1400	1900	920	400	4	50	2	2.6	16	9	2	1.5
	Sturmer Pippen	1400	1900	1000	440	3	35	3	4.8	24	9	3	1.5
	Mean	1400	1900	960	420	3.5	42.5	2.5	3.7	20	9	2.5	1.5

9. <u>Bacterial and fungal flora of the zones around T. versicolor</u>
   <u>infections</u>.

Apple sapwood appeared to be sterile. It was however,
frequently possible to isolate bacteria from the discolored tissue
which surrounded <u>T</u>. <u>versicolor</u> infections. The bacterial populations in
such tissues were not particularly diverse, usually being restricted to
a few species. Sometimes yeasts were isolated and on rare occasions
other fungal species. The species involved were not identified. Once
the tissues commenced to show obvious symptoms of white rot, <u>T</u>. <u>versicolor</u>
was often isolated entirely free of contamination by other organisms.

10. <u>Quantities of total extractable material and entractable</u>
    <u>phenolics in the discolored zone and adjacent sapwood</u>.

The discoloration of wood (including heartwood) has normally
been associated with an increase in the level of phenolic extractives in
such tissues. The discolored tissues were therefore expected to show
increased levels of extractable phenolics compared with sapwood.
Investigations were carried out initially to compare the efficiency
of two methods of extraction, the total amount of material extractable
and the proportion of phenolics in the extractable material. The material
used for these studies was obtained from <u>T</u>. <u>versicolor</u> infected
Cleopatra and STP limbs previously used for moisture, mineral and pH
determinations.

(a) Methods of extraction and quantities of material extracted.

The results (Table 5) showed that the hot and cold extraction
methods gave a very similar result both in terms of the total material

TABLE 5

Mean weight[†] of extractable material and total phenolics extracted
by hot and cold methods of extraction from different wood types.
(Results as mg./gm. oven dry wood 65°C.)

Methods of Extraction		Cleopatra Sapwood		Cleopatra Discolored Wood		STP Sapwood		STP Discolored Wood	
		T.E.M.[*]	T.P.[+]	T.E.M.	T.P.	T.E.M.	T.P.	T.E.M.	T.P.
Hot Extraction	Ether	4.81	0.77	3.82	0.19	4.40	0.66	3.26	0.21
	Methanol	66.00	26.17	37.95	7.29	48.44	18.74	29.52	6.97
	Total ether and methanol extracts	70.81	26.94	41.77	7.48	52.84	19.40	32.78	7.18
	0.1N NaOH extracts	49.93	0.16	44.40	0.34	56.40	0.16	45.53	0.37
	Total extractable material and phenolics (3 solvents)	120.74	27.10	86.17	7.82	109.24	19.56	78.31	7.55
Cold Extraction	Total methanol and 50% methanol extractable material and phenolics	68.24	26.42	40.91	8.65	49.40	19.90	33.60	8.53

[†] Each figure represents the mean of two extractions per sample of each wood type.

[*] T.E.M. - total extractable material.

[+] T.P.   - total phenolics determined by Folin Denis assay.

extracted and the total phenolics extracted [compare in Table 5 the total amounts of ether and methanol extractable material (hot extraction) and 50% methanol and absolute methanol extractable material (cold extraction)]. The initial ether extraction employed with the hot extraction to remove fats, oils and waxes did not appear to increase the efficiency of the methanol extraction. Ether was a poor solvent for phenolics. Sodium hydroxide extraction removed some additional material from both wood types, although less from the discolored wood. Little phenolic material was removed by NaOH, which indicated the efficiency of the methanol extraction. Because of its convenience, the cold extraction procedure was used in later work.

(b) Quantity of phenolics extracted.

Both the quantity of total neutral solvent extractable material and extractable phenolics declined in discolored wood compared with the adjacent sapwood (Table 5). It was noted however, that even after prolonged extraction with methanol and the refluxing treatment with 0.1N NaOH, that the discolored wood still retained most of its color. This and previous histochemical evidence indicated that the phenolics of the sapwood were probably polymerised and oxidised to an unextractable form in the discoloration process.

The figures can be compared with those of Hillis and Swain (1959) who found that the inner sapwood of plum contained a level of phenolics of 29 mg./g. dry wood compared with the 26.94 mg./g. found in Cleopatra and 19.56 mg./g. in STP sapwood. Undecayed plum heartwood contained a similar amount of phenolics as the inner sapwood. In apple however, it appeared there was a decline in extractable phenolics

in discolored wood. Tattar et al. (1971) have reported a fall in extractable phenolics in discolored wood around Fomes connatus decays in sugar maple.

(c) Fractionation of the extracted phenolics.

The differences in amounts of extractable phenolics between wood types led to investigation of possible differences between the nature of the phenolics extracted from the two tissues. The extracted phenolics (after lead acetate precipitation and recovery) were fractionated on a Sephadex column. The results for each wood type of the two varieties were very similar and only those for Cleopatra are shown in Figure 5.

The differences between wood types in the quantities of phenolics extracted, were reflected in the differences in the magnitude of the elution curves. The basic curves were similar however. Elution in both cases gave a dark red-brown front moving band followed by a brown to yellow trail. The general elution pattern was very similar to that for phenolics from discolored apricot wood (Somers and Harrison, 1967). The dark front band was difficult to separate from the yellow trail which appeared to show a separate peak. Increased column length and decreased column load led to the partial separation shown in Figure 5. The fractions were divided into three groups - (1) Group A consisted of the dark red-brown material; (2) Group B was intermediate between A and C and contained some of the red-brown material and the yellow material; (3) Group C was composed of fractions of the yellow trail. Preliminary thin layer chromatography with acetic acid and butanol-acetic acid-water confirmed that Group B contained material characteristic of Groups A

FIGURE 5

Elution curves of phenolics extracted from discolored
and normal apple sapwood (Cleopatra variety); following
fractionation on Sephadex G-25 in 50% aqueous acetone.
Absorbance measured at 450 mμ.
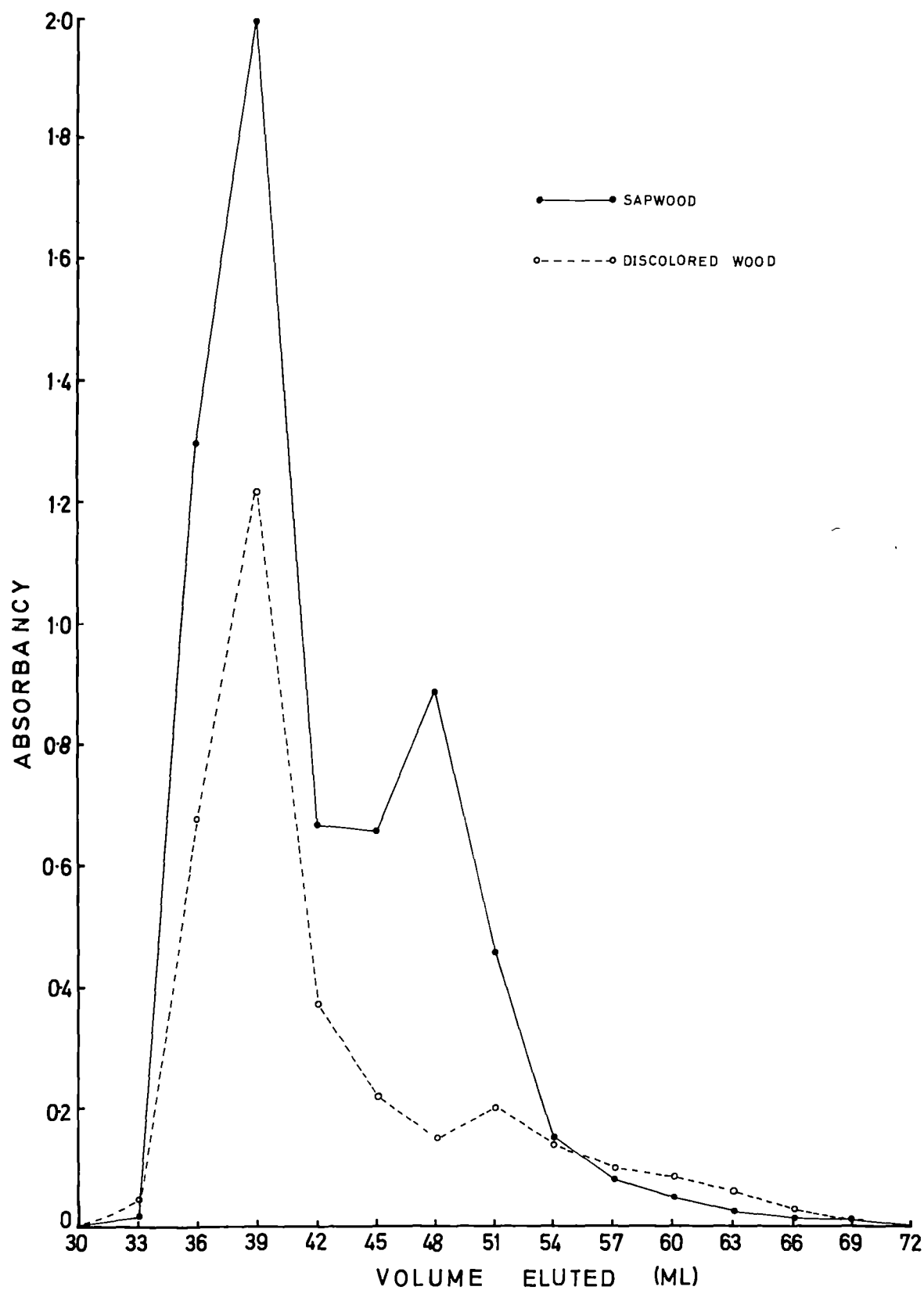
and C. With the sapwood extract, Group A showed little movement in either solvent and no separate spots were seen, while in Group C at least four discrete spots were seen. On chromatographic evidence, quercetin-quercitrin and phloridzin appeared to be the major mobile components. Group B showed the brown spot at the origin and 2 or 3 discrete spots. With the discolored wood extract Group B and C were reduced (1 spot in B, 2 spots in C) while Group A remained immobile. Thus, it appeared that the elution trail was probably composed of a number of different compounds of low molecular weight.

It appeared therefore, that there was no basic difference in the nature of the extractable phenolics in sapwood as compared with discolored wood, but the extractable phenolics were reduced in the latter, particularly with respect to the lower molecular weight components.

(F) The relationship between living cells and T. versicolor in the attack of apple sapwood.

Invasion of apple sapwood by T. versicolor resulted in a consistent series of changes in the wood in advance of penetration by the fungus. One significant result of these changes was that in the xylem tissue the xylem and ray parenchyma cells always appeared to die in advance of the fungal hyphae (i.e. the fungus did not penetrate

living cells). On the lateral margins of infections there was often a limited width of dead cells in advance of hyphal penetration (2-10 cells), but the extent of dead tissue was more extensive in advance of longitudinal penetration.

In the wood, the only situation in which _T. versicolor_ appeared to be actually colonising living cells was in the pith. There was a decline in starch and TTC reducing ability in the pith cells immediately in advance of the hyphae and sometimes nuclei also disappeared but generally cell death seemed dependent on hyphal penetration (Plate 28). Nuclei were sometimes seen in cells adjacent to those containing hyphae, and were normally present in cells within 2-4 cells of those which contained hyphae. In both young and old wood, hyphal penetration of the pith cells was accompanied by the formation in the cells of a variable quantity of extraneous material, which appeared similar in nature to that formed in discolored xylem and ray parenchyma.

In natural infections, where advance xylem discolorations were sometimes more extensive than normal, the pith column was also dead over long distances. Death of the pith was probably related to the isolating effect of the xylem discoloration, which would prevent translocation in the rays (Ziegler, 1964).
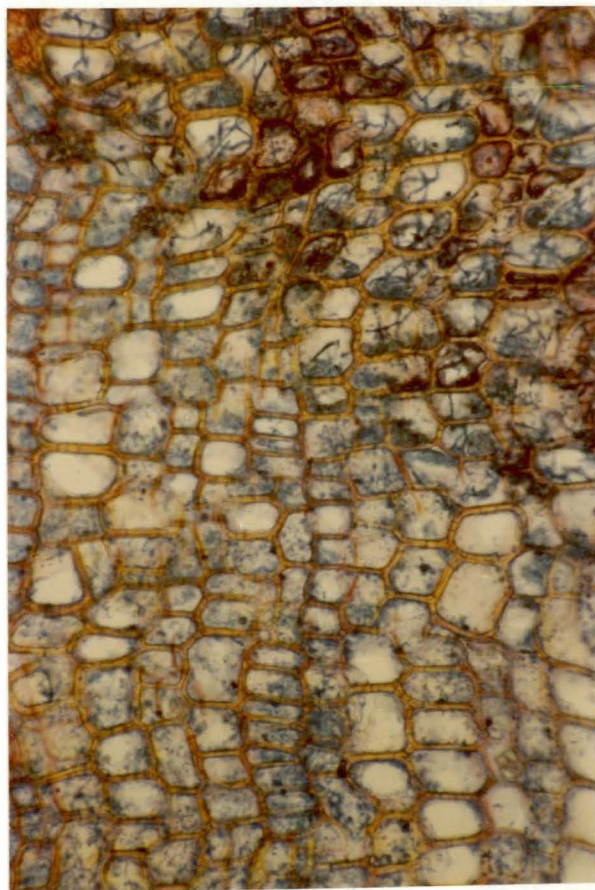
PLATE 28   Penetration of pith tissue by T. versicolor.
            Note hyphae in cells in upper half of plate
            and healthy pith cells in the lower half.
            Nuclei are present in cells almost adjacent
            to those penetrated by the fungus.
            (T. versicolor infection in 3-year-old STP
            limb. Cartwright's stain.   x 500)

(G) The role of discolored tissue in limiting T. versicolor infections.

Wounding or fungal invasion of living apple sapwood resulted in
the creation of a zone of discoloration beneath the wound or in advance
of the zone of fungal activity. The general nature of discoloration
indicated that it was a host reaction which possibly had a protective
effect in limiting the fungus and making the wood less susceptible
to decay. In summary, the main changes involved in discoloration were,
the loss of starch from the living ray and xylem parenchyma cells, cell
death and conversion of the cell contents into chemically inert material,
increases in moisture and mineral content in the zone of discoloration,
a change in the quantity and nature of the phenolic constituents and in
young wood, the formation of wound gum in the vessels.

To gain some indication of the overall effect of the discolor-
ation processes on the susceptibility of the wood to decay by T.
versicolor, in vitro decay tests were conducted. The only source of
sufficient discolored wood for decay testing was from around infections
in old and large limbs. In the first experiment, irregular sized
blocks of discolored wood and adjacent sapwood were obtained from the
Cleopatra and STP limbs previsouly used for the examination of other
properties. The results are shown in Table 6.

Considering the irregular nature of the material used, the
results were surprisingly uniform and indicated, at least in vitro, that
discolored tissues were more resistant to decay than the adjacent sapwood
used as a control. The results in Table 6 also indicated the possibility
of variations in the decay resistance of the discolored tissue between

TABLE 6

Mean percentage weight losses due to decay by T. versicolor, of two apple wood types in vitro.

	STURMER PIPPEN		CLEOPATRA	
	Sapwood	Discolored wood	Sapwood	Discolored wood
Limb Number				
1	52.7 (3)*	34.9 (2)	59.1 (8)	47.1 (3)
2	56.7 (3)	46.7 (3)	56.9 (11)	50.4 (6)
3	73.4 (3)	49.1 (3)	66.0 (13)	46.7 (10)
4	56.1 (12)	31.7 (10)		
Mean	59.7 (21)	40.6 (18)	60.7 (32)	48.1 (19)

* Figures in brackets represent number of samples of each wood type which contributed to the mean figure.

varieties and within limbs of the same variety.

In the second experiment it was possible to use uniform samples of discolored wood and sapwood from a single limb for decay testing. The results and statistical analysis are shown in Table 7.

The results were generally uniform and confirmed those of the preliminary experiment with regard to the relative decay susceptibility of sapwood and discolored wood in vitro. The large difference between the two strains in decay ability (significant at .1% probability) was not expected. Further comparisons would be required to show whether or not the strains that attack living apple trees have any consistent advantage in wood decaying ability, compared with isolates from decayed wood. There was no apparent difference in the ability of the two strains to cause dieback when inoculated into living apple trees. In this particular case, the differences in decay ability could be related to differences in growth habit of the two strains, which was independent of wood type. With D4, massive fungal growth occurred over the entire block and agar surface, while with DFP 12017 growth was scant and restricted to a sparse layer of mycelium covering the block.

Hossfeld et al. (1957), McNabb et al. (1959) and Hart and Johnson (1970) showed discolored sapwood of many hardwoods was generally more resistant to decay than sapwood of the same species, although exceptions did occur (Hart, 1964). The explanation for the increased decay resistance of discolored apple wood remains to be elucidated.

TABLE 7

Percentage weight losses of sapwood and discolored wood due to decay by two strains of T. versicolor and analysis of variance of the results.

Rep. No.	SAPWOOD		DISCOLORED WOOD	
	D4	DFP 12017	D4	DFP 12017
1	40.3	38.6	32.2	31.6
2	43.9	34.2	28.5	25.7
3	49.7	39.7	31.6	33.7
4	45.9	43.0	29.1	29.8
5	44.1	38.2	32.7	25.0
6	46.7	32.5	29.0	25.0
7	48.4	38.2	31.9	22.3
8	46.0	33.0	30.8	22.9
9	44.7	34.6	33.4	25.8
Mean	45.5	36.9	31.0	26.9

Analysis of Variance

Source	df.	Mean square	F
Replications	8	13.9	1.7
Treatments	3	588.7	71.8***
Strains (S)	1	368.0	44.9***
Wood type (W)	1	1353.0	165.0***
SxW interaction	1	45.1	5.5*
Error	24	8.2	

* Indicates level of significance –

    *** = .1%

     * = 5%

(H) Apple heartwood.

Observations made during this study indicated that under
Tasmanian conditions, heartwood formation in apple trees did not
occur until an age of 40-50 years or even older had been attained.
Study of apple heartwood was complicated by the cultural practices
employed with apple trees, in that it was often difficult to determine
whether discoloration of the sapwood was true heartwood or wound-
initiated discoloration.

Overall, only three examples of what could be considered genuine
heartwood, were examined. In all cases the heartwood appeared as a
small central column of brown discoloration in the limb. The color of
the heartwood appeared to be lighter than that of discolorations
resulting from wounding or T. versicolor infections. The heartwood
lacked living cells or starch and the cells contained a brown material
similar to that described in discolored wood. Only occasional small
gum plugs were observed in the vessels. A number of pH and moisture
determinations showed an average pH of 5.9 (6.10 in adjacent sapwood)
and an average moisture content of 84.4% (93.1% in adjacent sapwood).
Most limbs infected with T. versicolor contained no heartwood and
because of its rare occurrence it was not considered further.

SECTION II

Factors affecting discoloration and gum formation in apple
sapwood.

INTRODUCTION

The results obtained in Section I, showed that there was a consistent and definable response in apple sapwood to mechanical wounding and fungal invasion. From the preliminary work undertaken, the morphological and histochemical features of the response appeared very similar in each case.

In view of the paucity of quantitative results concerning the host response and the possible importance of discoloration and gum formation on the susceptibility of sapwood to Trametes versicolor, it was decided to study the wound response and some of the factors affecting it, in greater detail. The specificity of the sapwood response was examined in vivo and in vitro. The effect of season, age of wood, inoculation and method of inoculation with T. versicolor and age of wound on host response, were examined in vivo. The influence of ageing of wounds on their susceptibility to invasion by T. versicolor was also studied.

The concept of sapwood response (reaction) to wounding and fungal invasion, involves a range of reactions that occur in the wood,

leading to the death and discoloration of the tissue. The only readily measurable parameters of response in apple wood were the extent of gum formation and discoloration.

MATERIALS AND METHODS

(A) <u>Sterile and non-sterile wounds and sterile limb sections to study</u>
<u>discoloration and gum formation in response to wounding</u>

<u>In vivo</u>, non-sterile wounds were made by cutting off 2-5-year-old
branches of young apple trees. Sterile wounds were made by swabbing
the branch around the cutting point with absolute alcohol, flaming and
severing the branch with sterile secateurs. To maintain sterility, a
30 cm. length of sterile, cotton-wool plugged nylon tubing was pushed
over the cut stub. Sterile aluminium foil was used to make a cover of
the tube-branch junction, while the free end of the tube was pulled
down below the level of the wound, to prevent the entry of water.

Sterile limb sections were obtained from 2-year-old branches
of 5-year-old Sturmer Pippen trees growing in sand culture at the
University of Tasmania (details Section III). In the laboratory, the
cut ends of the branches were sealed with wax and the 12 to 20 cm.
lengths of limb immersed in 10% sodium hypochlorite for 10 minutes, for
sterilisation. After this treatment, the lengths were rinsed in three
changes of sterile distilled water and cut into 4 cm. sections with
sterile secateurs. Sections were stored in sterile dishes until placed
in incubation chambers.

(B) <u>Incubation of sterile limb sections</u>.

Limb sections in preliminary experiments were incubated in
sterile, 7 cm. diameter x 13 cm. high, glass jars. Sections were placed

in small beakers in the jars and the jars were either left dry or 50 ml. of sterile water was added to them. Jar lids were left loose or tightly sealed. For the incubation of limb sections on agar, 200 mls. of water agar was placed in the jars. Limb sections were inoculated or uninoculated with T. versicolor. Some sections were incubated under anaerobic conditions in Baltimore Biological Laboratories Gaspak Anaerobic Jars. Atmospheres in the anaerobic jars were at 100% R.H. due to the reaction creating anaerobic conditions. All treatments were incubated at 25°C for 30 days with 3 limb sections per jar and 2 replications per treatment. Relative humidity in the incubation room was 40-50%.

In experiments on the effect of humidity on the rate and extent of gum formation, limb sections were incubated either in closed dessicators over H_2SO_4 solutions of various concentrations (Handbook of Chemistry and Physics, 1944) or in atmospheres of constant flow and humidity, by pumping air through saturated salt solutions. Both methods gave satisfactory results.

For saturated salt solutions at each R.H. level, a slow-flowing compressed air supply was connected to a sterile cotton-wool filter and a series of four interconnected 250 ml. conical flasks, which contained respectively sterile water and three flasks of the required saturated salt solution. The inlet into each flask was fitted with a sintered glass filter submerged in the solution. The humidified air from the final flask passed into a sterile 1 litre incubating flask, which contained the limb sections. Saturated solutions of K_2SO_4(97.5% R.H. at 25°C, KH_2PO_4(96% R.H. at 25°C), KNO_3(92.5% R.H. at 25°C) and

$Ca(NO_3)_2 \cdot 4H_2O$(50.5% R.H. at 25°C) were used (Winston and Bates, 1960). Sterile water was used to provide an atmosphere of 100% R.H. Sections incubated at 50.5% R.H. were used as controls to check the occurrence of discoloration and gum formation in wood which dried rapidly.

Six limb sections were sampled at each of the 5 R.H. levels, after 5, 10, 15 and 21 days incubation. Three sections were used for the examination of starch depletion and extent of gum formation and three were used for moisture and pH determinations. Longitudinal sections (30μ) were cut and examined for gum formation by orcinol staining, and for starch with I-KI solution (page 43). The limb sections for moisture and pH determinations were halved transversely, one half being used for moisture determination and the other half for pH determination, as previously described (page 40). Control limb sections were taken at the beginning of the experiment for pH and moisture determinations. Blocks used in experiments were taken during December-April but other trials indicated that season had no effect on response in vitro.

(C) Material for the study of the effect of age of wood on gum formation

Four healthy 40-50-year-old bearing STP trees, growing on a property at Cygnet, were available for the experiment. In winter 1970, four main scaffold limbs per tree were cut off within 20-40 cm. of the main stem. The cut surfaces were trimmed with a knife, left uncovered and harvested as required over the next eight months. Orcinol, I-KI, TTC and Cartwright's staining procedures were used on longitudinal sections as previously described (page 43).

In February 1971, similar limbs were selected at random from the same trees, for carbohydrate and mineral analyses (Methods in Section III). A disc 3 cm. wide was cut from the limb at a point approximately 30 cm. from the main stem. This cutting point was chosen as being equivalent to the point of wounding on the limbs in the experiment, and a 3 cm. disc gave adequate material for analysis. The top of the limb was marked prior to its removal. For transverse sampling, samples were taken across two diameters between opposite pairs of cardinal points using the top of the limb as a reference. One cm. square blocks were excised inwards from the cambium and the four blocks from the same depth were pooled to give a composite sample for each depth. The central block containing the pith was taken as a single sample and the number of samples depended on the size of the limb.

(D) <u>Material and experimental design for studying the affect of season on the rate and extent of gum formation and other cellular changes under wounds in vivo.</u>

The trees selected were healthy 40-year-old bearing STP trees, growing on a uniform, well drained slope at Castle Forbes Bay. The trees comprised a compact block of 250 trees, with a row spacing of 4.9 x 4.9 m. Most trees had a trunk girth, at 20 cm. above ground level, in the range 68 ±7.5 cm., and 36 trees were selected at random from the trees in this girth range. These trees were in turn randomly divided into two series of 18 trees. Series 1 trees were used for wounding and inoculation studies, while series 2 trees were used for samples for

chemical analyses. Samples for chemical analyses were not taken from series 1 trees to avoid removing large numbers of limbs, in addition to those removed in wounding and inoculation experiments. Analyses of leaf samples from the 36 trees showed no significant differences between the two series for levels of five major (N, K, Ca, P, Mg) and three minor (Mn, Fe, Cu) elements. Analyses of wood and bark samples from series 2 trees were therefore considered representative of the nutritional status of series 1 trees.

Wounds and inoculations were made in (a) July (winter) - representative of the dormant condition, (b) December (summer) - representative of high physiological activity and (c) April (autumn) - representative of the semi-dormant state. All wounds and inoculations were made by cutting off 5-year-old branches in the diameter range 1.2-1.4 cm. and the nearest bud was normally 2-4 cm. below the cut surface. Plate 29 shows the type of wound used in this study.

Three wounds per tree were made at random at the beginning of July (1969), December (1969) and April (1970). Wounds were harvested on the 15th and 30th of these same months and 120 days after wounding. Control samples were taken at the time of wounding in each season, to check starch=distribution. Thus, wounds were made in three seasons and four ages of wound were studied, T0 - time of wounding, T15 - wound 15 days old, T30 - wound 30 days old and T120 - wound 120 days old. Any bark .. symptoms were measured at harvest and the limbs were cut off 5 cm. below the point of original wounding, fixed in FAA and stored in 70% alcohol.

PLATE 29   An uninoculated wound in the 5-year-old wood
of a limb on a 40-year-old STP tree.
(Photographed December 1969).

The extent of discoloration beneath a wound was measured by splitting the limb longitudinally through the pith, in the plane at right angles to the axis of the tree. The depth of discoloration below the wound surface, was measured at the central point of the xylem between the cambium and the pith on each side of the section and the two results averaged. Longitudinal sections 1-3 cm. long and 30μ thick were cut in the same plane and examined for gum, starch and fungal hyphae (methods, page 43). Measurable parameters were the extent of gum formation and the depth below the surface at which gum formation started. Preliminary measurements showed that in wounds in young wood, the longitudinal extent of gum formation was approximately the same across the entire xylem radius, despite the variability of the depth below the wound surface at which gum formation started. Measurements for these parameters were therefore standardised at the midpoint of the xylem between cambium and pith on each side of the section and the two figures for each section averaged.

Samples of uninoculated wood, corresponding in age to the inoculated samples, were taken for carbohydrate, nitrogen and phosphorus analyses, periodically through the seasons. Four pieces of 5-year-old wood per tree were selected at random at each sampling and pooled to give a composite sample per tree. Samples were placed in a cool chamber and, on return to the laboratory, bark and wood separated and the tissues dried at 65°C. Tissues were prepared and analysed by methods given in Section III.

(E)   <u>Material and experimental design to study affect of inoculation</u>

<u>with T. versicolor, method of inoculation, age of wound and season</u>

<u>on gum formation and fungal penetration.</u>

This experiment was run concurrently on the same series of
trees as used in (D) with the same seasonal treatments, ages of wound
and wound size. Wounds were inoculated with T. versicolor by the method
described in Section I.

Two wounds per tree were made on randomly selected limbs on the
first day of each designated month. One of these wounds was inoculated
on the 15th (T15) and the other on the 30th (T30) of the same month.
A further wound was made on the 15th of each month and inoculated
immediately (T0). This system of wounding and inoculation within season,
was designed to minimise the effect of any physiological changes that
occurred in the wood between the time of initial cutting and final
inoculation, and the assumption was made that the age of wound was the
only effective treatment. The experiment was a 3 x 3 factorial with
18 trees.

Longitudinal sections were cut and stained for gum, starch and
fungal hyphae as for uninoculated wounds. The same parameters were
measured as in the uninoculated wounds and the length of hyphal pene-
tration was also measured. Results in Section I showed that in
inoculations of this type, maximum hyphal penetration occurred in the
inner secondary xylem, and to allow direct comparison between the two,
this measurement was standardised with those of the extent of gum
formation and the depth of gum formation at the mid-point of the

secondary xylem. All observations and measurements were made on a Zeiss RA Research Microscope with a calibrated stage.

(F) Statistical analyses.

Parameters measured in (D) were analysed with main effects of season of wounding and trees, and season x tree interaction was used as an error term. Analyses of variance of the parameters in (E) were conducted with main effects of season of wounding, age of wound and season x age interaction. Variances due to trees, age x trees, season x trees, season x age x trees were pooled to give an error term. In each case the homogeneity of variances composing the error term were tested using Bartlett's test (Steel and Torrie, 1960). In all cases analysed, variances were found to be homogeneous.

The main treatment effects in (D) and (E) were partitioned into single degree of freedom orthogonal comparisons to separate statistically significant effects. The main interaction in (E) (season x age) was also partitioned when significant. A straightforward partitioning based on expected results was used.

OBSERVATIONS AND RESULTS

(A) In vitro wound response of sterile apple limb sections.

Discoloration and gum formation occurred under sterile wounds
in vivo and under wound surfaces on limb sections in vitro. Inflicting
and maintaining sterile wounds in the field was difficult, and wounds
were frequently contaminated by bacteria. However, sufficient
remained sterile to prove that discoloration and gum formation occurred.
Discoloration and gum formation also occurred under sterile conditions
in vitro, where it was possible to more effectively control sterility.
As a general observation, the amount of discoloration which occurred in
a given time was less extensive and of a lighter color under sterile
wounds than under non-sterile wounds.

The evidence appeared sufficient to show that discoloration and
gum formation occurred in the absence of micro-organisms, presumably in
response to physical stimuli. This observation is in agreement with
Lorenz (1944), Grosclaude (1966b), Barcukova (1967), Shigo (1967a) and
Sucoff et al. (1967). The extent of the response under sterile conditions
in vivo was not investigated further, but a number of experiments with
sapwood were conducted in vitro, to examine gum formation and discoloration
and factors affecting their occurrence.

The complementary factors of aeration and desiccation have been
reported to influence discoloration and gum formation in sapwood
(Literature Review). As an initial study, a wide range of treatments
were tested on limb sections of young apple wood to determine their
affect on the ability of the wood to form gum. The results are
presented in Table 8.

TABLE 8

Occurrence of discoloration and gum formation in sterile limb sections under a range of incubation conditions.

(All limb sections incubated at 25°C for 30 days.)

Group No.	Incubation treatment of blocks	Dis- coloration	Gum formation
1 Limb sections dried at 65°C for 24 hours	(1) Water agar	-*	-
	(2) Loose lid sterile jar	-	-
	(3) Saturated atmosphere, sealed jar	-	-
	(4) As in (3), inoculated	-	-
2 Limb sections autoclaved at 15 p.s.i. for 10 min.	As in (1), (2), (3) and (4) above	-	-
3 Sterile limb sections in aerobic atmos- phere	(1) Water agar	-	-
	(2) Water agar, inoculated	-	-
	(3) Loose lid sterile jar	+	++
	(4) As in (3), inoculated	+++	+/-
	(5) Saturated atmosphere, sealed jar	+	+
	(6) Saturated atmosphere, sealed jar, inoculated	+++	+/-
	(7) Dry sealed jar	+	+
	(8) Dry sealed jar, inoculated	+++	+/-
4 Sterile limb sections in anaerobic atmosphere	(1) Water agar	-	-
	(2) Anaerobic jar, saturated atmosphere	+/-	
	(3) Anaerobic jar, saturated atmosphere, inoculated	+/-	-

*- No gum formation or discoloration.

+/- Minor amount of gum formation or discoloration.

+ Small amounts of gum in outer secondary xylem or discoloration extends across the entire diameter of the xylem, both to a depth of several mm. below the surface.

++ Gum more extensively but irregularly distributed across the xylem.

+++ Extensive discoloration extending through most of the block.

Killing of the living limb sections by rapid drying (Group 1) or autoclaving (Group 2) effectively prevented discoloration or gum formation under a range of incubation treatments. Incubation of limb sections under anaerobic conditions gave a similar result (Group 4). Slight discoloration of the cut ends of limb sections in some anaerobic treatments (Table 8) probably occurred prior to the establishment of a completely anaerobic environment. Growth of T. versicolor did not occur under anaerobic conditions.

The only treatment group in which discoloration and gum formation were observed, was in living limb sections incubated in aerobic atmospheres (Group 3). Sections incubated on water agar had a film of water covering the cut ends and under these conditions no discoloration or growth of T. versicolor occurred, apparently because of the poor aeration. The most extensive discoloration and gum formation occurred in limb sections incubated in saturated atmospheres (free water absent from wound surfaces) or under conditions in which slow drying occurred (e.g. loose lid jars). Limb sections inoculated with T. versicolor under similar conditions, were rapidly colonised and killed by the fungus with little or no gum formation (Table 8). Gum formation and discoloration were normally restricted to within 2-5 mm. of the cut ends of uninoculated limb sections.

The results from these treatments emphasised that discoloration and gum formation were a response of living tissue, and confirmed that aeration and desiccation were two important factors influencing discoloration and gum formation. The fact that gum formation and discoloration occurred in aerobic environments even at 100% R.H., but not in

anaerobic environments at 100% R.H., indicated the importance of oxygen to the process of wound response.

Following the results obtained in preliminary experiments, the rate and extent of gum formation, the rate of drying of limb sections and changes associated with discoloration and gum formation were investigated in sterile limb sections, incubated at a range of humidities. The results are presented in Table 9.

The moisture content of blocks decreased with increasing length of incubation even in blocks incubated at 100% R.H. The small amount of drying that occurred at 100% R.H. probably arose from the constant outflow from the incubating flask but this source of error also occurred in the other humidity treatments. Control limb sections dried rapidly to a very low moisture content.

The pH of the blocks showed an initial fall after incubation commenced, but after 10 days of the pH appeared to show a rise in all treatments except the controls. The fact that the pH decreased with discoloration and gum formation under sterile conditions, provided additional evidence that the host reaction to wounding resulted in a real fall in pH and that this was not an artefact due to microbial activity.

Plate 30 shows the type and extent of discoloration that occurred in the limb sections. Due to its irregular extent, it was not possible to measure the length of discoloration. Gum formation was first visible at 5 days in the 100%, 97.5% and 96% R.H. treatments and at 10 days in the 92.5% R.H. treatment. In the treatments at high humidity, the quantity of gum gradually increased up to 15 days, after

TABLE 9

Rate of drying, change in pH and rate and extent of
gum formation in sterile limb sections incubated at
a range of relative humidities (each figure mean of
3 replications).

Parameter	% R.H.	Time of incubation (days)				
		0	5	10	15	21
Moisture content of blocks (% dry wt. basis)	100.0	128.3	122.1	121.5	117.6	112.0
	97.5	128.3	111.8	113.1	113.6	107.3
	96.0	128.3	118.2	115.0	108.0	105.0
	92.5	128.3	106.4	101.4	104.0	98.4
	Control (50.5% R.H.)	128.3	44.7	20.0	11.1	9.0
pH within blocks	100.0	6.14	5.76	5.70	5.80	5.83
	97.5	6.14	5.91	5.80	5.85	5.98
	96.0	6.14	5.84	5.84	5.88	5.88
	92.5	6.14	5.70	5.80	5.80	5.85
	Control	6.14	5.88	5.86	5.88	5.88
Rate and extent of gum formation	100.0	−	+*	++	+++	+++
	97.5	−	+	++	+++	+++
	96.0	−	−	+	++	+++
	92.5	−	−	+	+	++
	Control	−	−	−	−	−

*
 +   small amounts of gum in outer secondary xylem.

 ++   gum more extensively but irregularly distributed across the xylem.

 +++ vessels across the entire diameter of the xylem occluded with gum
      but gum only extended a few mm. beneath the cut surface.
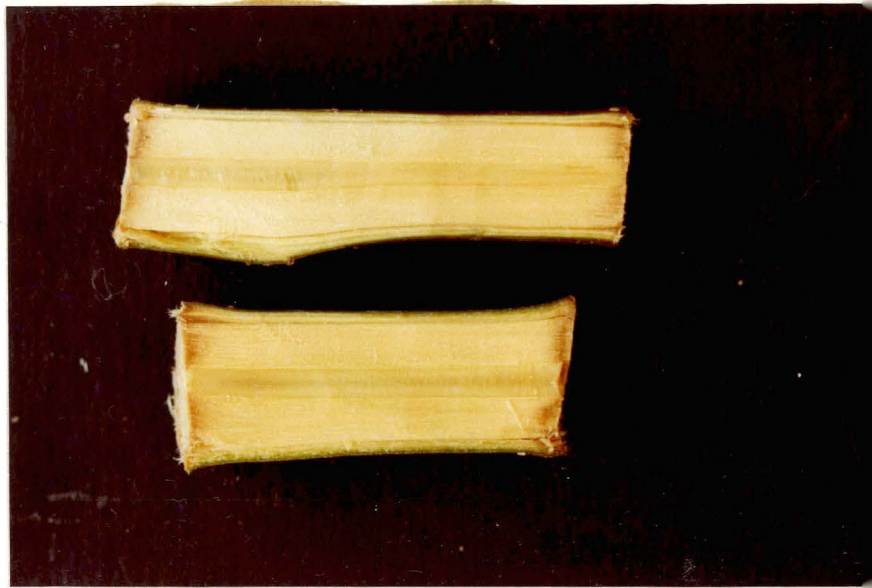
PLATE 30   Discoloration under the cut ends of limb
            sections incubated at 97.5% R.H. for
            21 days.

PLATE 31   The distribution and quantity of gum formed
            under the cut end of a limb section
            incubated at 97.5% R.H. for 21 days.   The
            photograph shows half the width of a
            longitudinal section cut through the pith
            and the pith can be seen on the left.
            (x 32)

which time no further increase was observed. The rate of gum formation and its extent were less at 92.5% R.H. No gum formed in the control limb sections incubated at 50.5% R.H. Other experiments showed that in limb sections incubated at less than 90% R.H., no discoloration or gum formation occurred, because of the high rate of drying and consequent death of cells.

Typically, gum formed in a zone 1-4 mm. wide and started 1-3 mm. beneath each cut surface of the limb section. Initial gum formation always occurred in the youngest xylem tissue. Gum formed closest to the cut surface in the oldest and youngest xylem and furthest below the surface in the mid secondary xylem. Plate 31 shows the type of distribution normally observed. The distribution was similar to that observed under wounds and fungal inoculations in young wood. The form of the gum was generally as plugs or irregular globules (Plate 31). It was initially proposed that the longitudinal extent of gum formation in the xylem be used as an estimate of the amount of gum formed, but the distribution and the irregular intensity of gum formation prevented this. Consequently quantities were assessed and rated (Table 9). There seemed little difference in the amount of gum in limb sections incubated at 100, 97.5 and 96.0% R.H.

Another feature which paralleled the formation of gum in the limb sections was the depletion of starch from the cells. With time, starch disappeared from the cells beneath the wound surfaces of the limb section and decreased in amount inwards from the cambium. Initially, there was plentiful starch in the pith and ray cells, but after 21 days little was left in the rays, although the pith still contained some starch.

While gum formation presumably accounted for some of the depletion, an additional factor, callus formation, was probably significant. Callus outgrowths proliferated from the vascular cambium at the cut ends of the blocks, in the high humidity treatments. Callus outgrowths were first noticeable after 10 days and continued to develop until the final harvest (21 days).

The amount of gum formed was generally fairly limited, probably because of the depletion of carbohydrate resources and other materials in the small limb sections.

The results obtained in these experiments showed that discoloration and gum formation in apple sapwood occurred in response to wounding and in the absence of micro-organisms. The general cellular changes that occurred with discoloration and gum formation under sterile conditions, appeared identical with those that occurred under non-sterile wounds and around fungal decays. Thus, the reactions of the sapwood involving discoloration and gum formation were a non-specific host response to external stimuli.

(B) Factors affecting the wound   response, particularly gum formation, in vivo.

Discoloration and gum formation normally occurred in response to both abiotic and biotic factors. In the case of some factors affecting response, there seemed no advantage in studying the response in the absence of the biotic element. A necessary assumption for such studies was, that the general level of microbial influence (other than in the case of a pathogen) in wound response, was uniform. With this assumption

and the difficulty of keeping wounds sterile in vivo, investigation of a number of factors affecting discoloration and gum formation were carried out on non-sterile wounds.

1. The age of wood.

Early in these studies it was observed that gum formed in considerable amounts in the vessels around wounds and fungal inoculations in young wood (up to 5-years old) but that around infections in old wood (20-years or older) gum formation was very sparse (compare Plates 22 and 23). The relationship between the age of wood and its ability to form gum was investigated in branch stubs of 40-50-year-old scaffold limbs of STP trees. Plate 32 shows one of the stubs at the time of commencement of the experiment (August 1970). The average diameter of the stubs was 8.0 cm.

TTC staining of control discs at the time of cutting showed that the ray and xylem parenchyma were alive across the entire limb radii, and that the pith cells were also alive. The greatest intensity of staining with TTC was in the 1.0 cm. of xylem adjacent to the cambium and staining decreased across the xylem towards the pith. The intensity in the pith increased slightly compared with the inner·sapwood.

Starch was present in the ray, xylem and pith parenchyma. By visual observation, the greatest amount was present in the cells in the outer xylem, with the pith cells showing a higher concentration per cell than the inner sapwood xylem and ray parenchyma. While this distribution was not verified by analysis at the commencement of the experiment, later samples taken from equivalent limbs on the same trees (February 1971),

PLATE   32   An example of the large branch stubs
used to study the relationship between
the age of wood and the ability of the
wood to form wound gum. (Photographed
at the time of wounding, August 1970.)

showed this pattern (Table 10). The level of soluble sugars did not appear to alter markedly across the xylem, while in the centre sample which contained the pith, the level appeared to rise. In one case the level in the pith sample decreased compared with the inner xylem, but the decline in soluble sugars was compensated for by a rise in starch level. The starch and hemicellulose contents both declined across the xylem, but tended to increase in the sample which contained the pith. The increased carbohydrate level in this sample probably occurred because of the higher proportion of living cells, as well as a rise in the concentration of carbohydrates per cell. Nitrogen and phosphorus analyses of the same samples (Table 11), showed a similar trend in concentration of these elements in the xylem and pith.

Sampling of single limbs at 1-2 month intervals revealed that discoloration, extending a few mm. below the cut surface, was noticeable after 30 days. Gum was also visible after 30 days. It took the form of small viscous globules in the vessels of the youngest xylem tissue. Later samplings showed that this pattern of development, with increased intensity and extent of discoloration, continued, especially in the inner secondary xylem (Plate 33). In xylem, ray and pith parenchyma immediately beneath the wound surface, death and discoloration occurred by disorganisation and aggregation of starch granules within the cells but deeper in the discolored zone, cells first lost their starch and then discolored. Gum formation remained restricted to the outer 0.5-1.2 cm. of youngest xylem tissue, normally commencing approximately 0.5 cm. below the wound surface and extending 1.5-2.5 cm. in depth. It mainly took the form of globules, but gum plugs were present to a lesser extent.

TABLE 10

Levels of component and total carbohydrate resources
(mg./g. dry matter) across transverse sections of
scaffold limbs of 40-50-year-old STP trees. (Samples
taken February 1971.)

Limb No.	Distance from cambium (cm.)	Soluble sugars	Starch	Hemi-cellulose	Soluble sugars & starch	Total carbo-hydrates
1	0-1	15.85	39.18	279.86	55.03	334.89
	1-2	12.08	27.50	288.97	39.58	328.55
	2-3	12.93	19.46	255.96	32.39	288.35
	3-4	13.38	12.36	247.25	25.74	272.99
	4-5 (centre)	18.24	24.33	258.80	42.57	301.37
2	0-1	14.61	33.21	270.74	47.82	318.56
	1-2	14.97	25.54	268.70	40.41	309.11
	2-3	14.07	20.89	240.24	34.96	275.20
	3-4 (centre)	21.35	21.91	246.56	43.26	289.82
3	0-1	15.27	44.04	270.74	59.31	330.05
	1-2	15.77	34.42	255.96	50.19	306.15
	2-3	14.79	15.49	261.83	30.28	292.11
	3-4 (centre)	9.46	27.50	252.92	36.96	289.88

TABLE 11

The nitrogen and phosphorus contents (parts per million dry matter) across transverse sections of scaffold limbs of 40-50-year-old STP trees. (Samples taken February 1971)

Limb No.	Distance from cambium (cm.)	Nitrogen	Phosphorus
1	bark	4080	555
	0-1	840	198
	1-2	580	91
	2-3	540	72
	3-4	540	72
	4-5 (centre)	560	97
2	bark	3660	730
	0-1	1380	160
	1-2	840	79
	2-3	1080	103
	3-4 (centre)	1080	79
3	bark	4020	730
	0-1	900	185
	1-2	780	91
	2-3	570	79
	3-4 (centre)	720	88

PLATE 33   The distribution of discoloration under a
           large STP branch stub, seven months after
           wounding. (Photographed February 1970.)
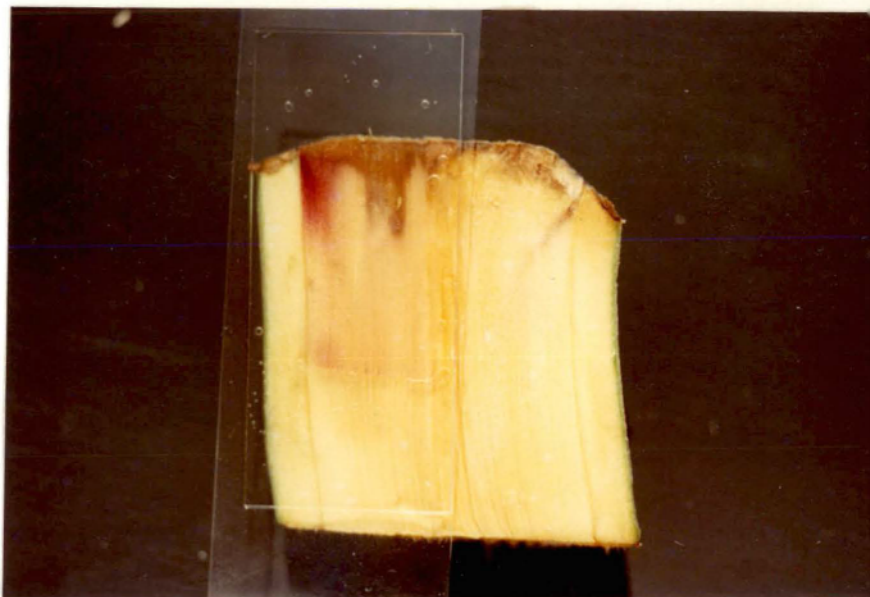
PLATE 34   The relationship between the age of wood
           and its ability to form gum. An orcinol
           stained, longitudinal section cut from
           the stub was superimposed over the stub.
           The red-purple stained gum was restricted
           to the outer 0.5-1.0 cm. of the wood.
           (Photographed December 1970.)

FIGURE 6

The distribution of discoloration and wound gum in a branch
stub of a 40-50-year-old STP limb, seven months after wounding.
(Wounded August 1970, drawn to scale.)

WOUND
SURFACE

DISCOLORED
WOOD

ZONE OF
WOUND GUM

BARK

SAPWOOD

PITH
COLUMN
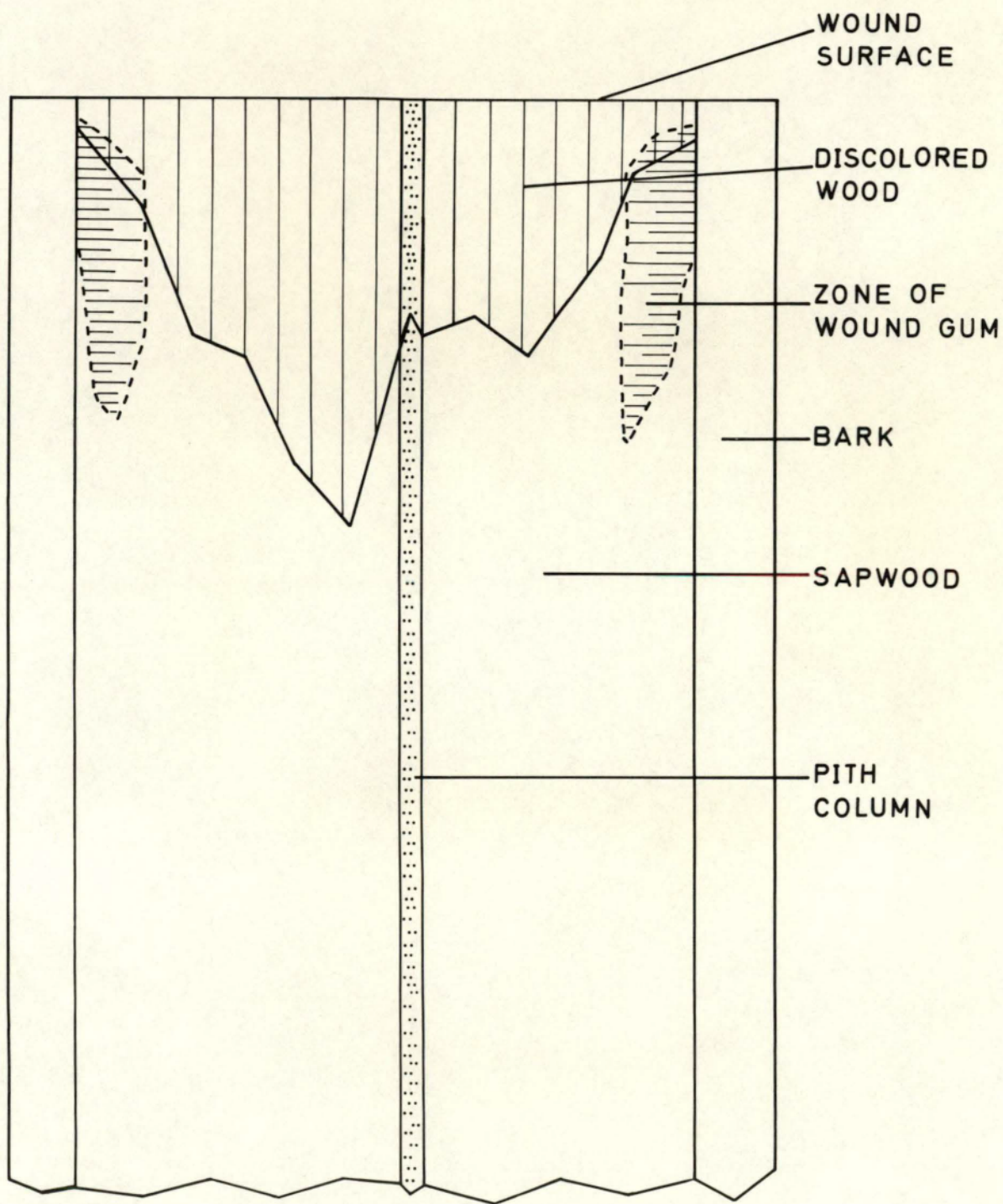
In the outer xylem, the patterns of discoloration and gum formation over-
lapped to some extent but gum formation occurred in the sapwood-
discolored wood transition zone. Figure 6 and Plate 34 show the
distribution of gum and discoloration in branch stubs after seven months.

In only half of the stubs examined, did the extent of discolor-
ation remain as restricted as that illustrated in Plate 33. The other
half developed extensive columns of discoloration in the inner xylem
tissue. Several were infected with T. versicolor and showed obvious
signs of white rot. Even in these cases of more extensive discoloration,
gum formation was still restricted to the youngest xylem.

Thus, the only extensive and intensive occlusion of vessels by
wound gum occurred in the youngest wood and gum was absent from most of
the xylem vessels. Counts of annual increments proved difficult due to
the characteristically small increment size (1-2 mm.) in the outer xylem.
As far as it was possible to determine, it appeared that gum formation
occurred in tissues in the age range 0-8 years, but tissues up to 12-15
years of age retained limited gum forming ability.

The investigation showed that apple sapwood can remain alive for
at least 40-50 years without heartwood formation. The results also
confirmed the observation that little gum formation occurred in the
vessels around infections in wood older than 15-20 years. With ageing
the xylem and ray parenchyma cells lost their ability to form gum. In
old limbs it was possible to make a distinction at about 12-15 years of
age between younger wood, whose cells produced large amounts of gum and
older wood, whose xylem and ray parenchyma had only very limited gum
forming ability. The decline with age in the ability of cells to

synthesise gum, appeared to parallel decreases in the levels of carbo-
hydrate resources, nitrogen, phosphorus and vital staining ability
across stems. Relative gum forming ability with age, within the young
wood category, was not investigated further.

2. Effect of season on gum formation and other cellular
changes under wounds in young apple wood in vivo.

Differences in the rate and amount of discoloration and gum
formation may influence penetration by T. versicolor in young apple sap-
wood. Seasonal effects, and particularly the directly associated
physiological activity of the tree, could influence the rate and amount
of gum formed, independently of other experimental treatments. The
effect of season on gum formation and other cellular changes was
investigated in non-sterile wounds, uninoculated with T. versicolor.
Results will be presented in relation to time after wounding, with
reference to seasonal differences.

(a) Time 0 (T0) – time of initial wounding.

No gum was present in healthy sapwood at the time of wounding.
Starch was present in the ray and xylem parenchyma cells at all times
of the year, although less was present in December (summer) as compared
with July (winter or April (autumn). The pith was densely packed with
starch in July and April but contained little starch in December.

(b) Time 15 (T15) – fifteen days after wounding.

At all seasons of the year changes in the tissue beneath the
wound surface were noticeable after 15 days. Macroscopically, the
first few mm. below the wound surface were discolored a light-brown,

Cartwright's stain revealed occasional hyphae extending 1 or 2 mm. into vessels but there was no general colonisation of the wound surface by fungi.

There appeared to be a general tendency for the starch to decrease in the 1-2 cm. of wood below the wound surface, although this was not verified by measurement. A zone of ray and pith parenchyma cells at the wound surface (usually 1-10 cells wide but sometimes 20-30), retained the starch in its normal granular form. Below this zone, some cells showed signs of the disorganisation and aggregation of starch granules within the cells, as previously described (Plate 21). Cells below this zone appeared normal.

Gum formation was minimal at T15 at all times of the year. In a few stubs some gum was present but in most no gum could be seen. When present it took the form of small irregular globules, present only in the first 1-5 vessels in the youngest secondary xylem (Plate 35).

(c) Time 30 (T30) - thirty days after wounding.

At 30 days the macroscopically visible discoloration was more extensive than at T15, although it tended to be irregular in depth. Differences in extent between seasons were not marked at this stage.

Microscopically, cellular changes were much more marked. Cartwright's stain revealed a situation similar to that at T15, with only a few scattered hyphae making limited penetration into the vessels. The general decrease in starch below the wound surface was more marked but an irregular layer of cells at the wound surface still retained their starch. Beneath these cells was a zone of ray and pith cells (10-40 cells wide), in which disorganisation and aggregation of starch
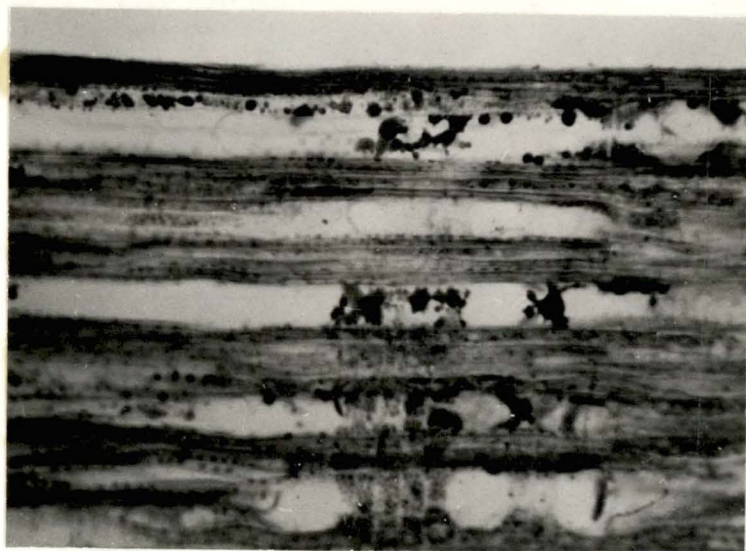
PLATE 35   The initial gum formed in the youngest vessels
            of the secondary xylem beneath a wound in 5-
            year-old STP wood. Note the small irregular
            globules of gum. (Wound 15 days old, July
            1969, x 150.)

granules occurred (Plate 21). The extent of this zone appeared greatest in December. Below this zone was a more limited zone of cells which showed gradual loss of starch and death of cells. Gum formation occurred in this zone.

Much more gum had formed by T30 compared with T15, in all seasons, although the depth below the wound surface at which it commenced to form varied with the season (Table 12). At all seasons, gum was present across the entire radius of the xylem from cambium to pith, with apparent occlusion of most vessels. The amount of gum at T30 appeared to be greater in December, when the vessels were more extensively occluded, than in July or April. Likewise there was more gum beneath wounds at T30 in April than in July (Plate 36). It was not possible to measure the extent of gum formation at T30 because of the irregular lower margins of the gum zone. Gum formation started in the youngest xylem, and was closer to the wound surface and greater in extent in that area. Gum formation also tended to be closer to the surface in the primary xylem and older secondary xylem than in the mid-secondary xylem (Plate 36). The form of the gum at T30 also varied with season. In July, particularly, the gum took the form of many discrete globules with few gum plugs, but in December and April larger globules and plugs were evident (Plate 36).

(d) Time 120 (T120) - one hundred and twenty days after wounding.

The pattern established at T30 had become more extensive and better defined after 4 months. The discoloration resulting from wounds in December was significantly greater in extent than that which resulted from wounding in July or April (Table 12). The discoloration often tended to be mottled in appearance, and in December, particularly, the

TABLE 12

Mean depth below wound surface gum formation starts in
uninoculated wounds 30 and 120 days old, and the mean lengths
of discoloration and gum formation in uninoculated wounds
120 days old, in wounds made in three seasons. [Each figure
is the mean of 18 observations, analyses of variance
Appendix I (a)(b)(c).]

No.	Parameter	Month of wounding			Orthogonal comparisons
		JULY(J)	DECEMBER(D)	APRIL(A)	
1[†]	Mean depth below surface gum starts in wounds 30 days old (mm.)	1.7	4.5	1.9	D vs J,A*** J vs A n.s.
2	Mean length of discol. below wounds 120 days old (mm.)	4.6	9.0	3.6	D vs J,A*** J vs A*
3[†]	Mean depth below surface gum starts in wounds 120 days old (mm.)	2.1	4.1	1.8	D vs J,A*** J vs A n.s.
4	Mean length gum formed in wounds 120 days old (mm.)	5.1	9.5	3.3	D vs J,A*** J vs A**

Asterisks indicate level of significance.

     *** = 0.1%   ** = 1.0%   * = 5.0% (also applies for
                                          Tables 14, 15)

    n.s. = not significantly different.

    †   1 and 3 not significantly different within each season.
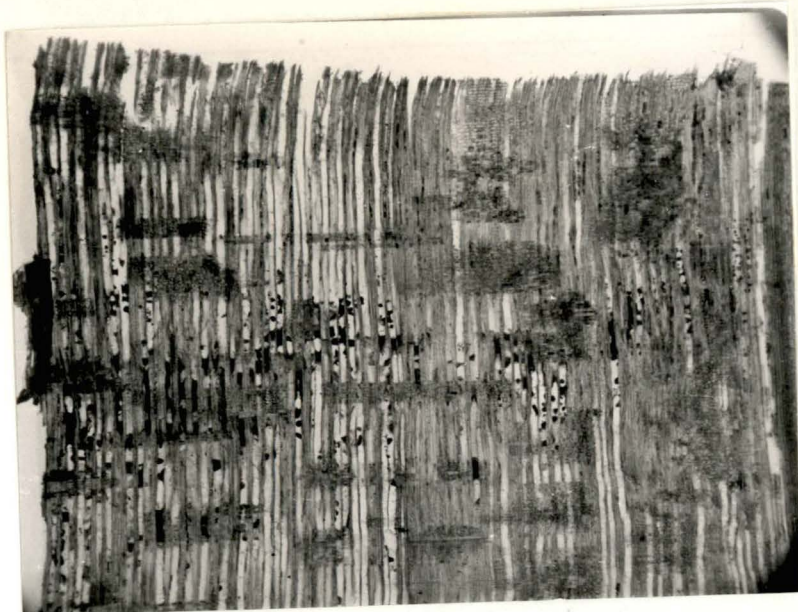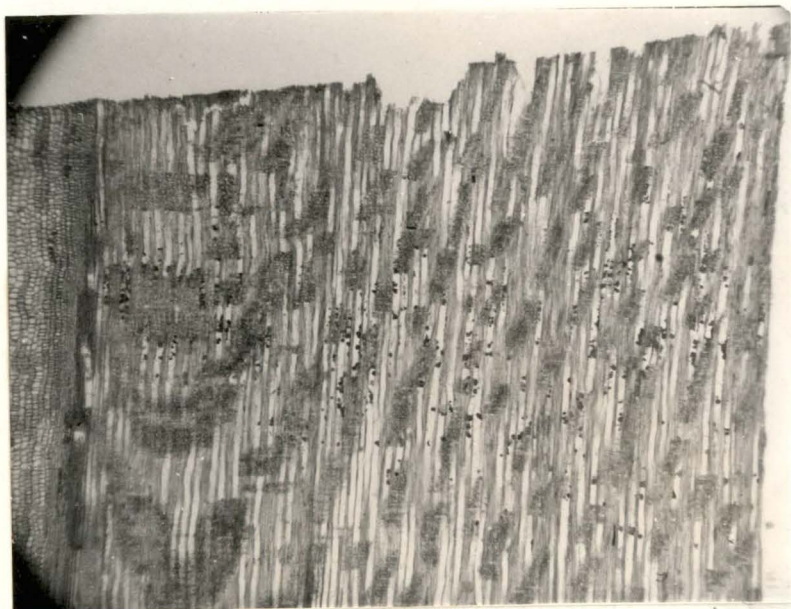
PLATE 36   Quantity and distribution of gum beneath
            30 day old wounds in 5-year-old STP wood.
            Wounds were made in July 1969 (top)
            and April 1970 (bottom). Note the
            distribution of the gum and differences
            in the morphology of the gum in July and
            April. Photographs show half the width
            of a longitudinal section cut through the
            pith. (x 32)

border between healthy and discolored wood was frequently marked by a darker zone of discoloration (Plate 37). A greater number of single fungal hyphae were present in the wounds at T120, although there was no general colonisation and most only penetrated a few millimetres.

Some starch filled cells existed at the wound surface in most wounds, even at T120, although this was more common in wounds made in July and April (Plate 38). Swarbrick (1926) observed that starch filled cells existed for up to 18 months at the surface of wounds made on apple, plum, sycamore and rhododendron. Beneath the starch filled cells was a zone of dead and discolored cells, in the lower part of which gum was present. The extent of the zone of dead parenchyma was dependent on the season of wounding. In wounds made in December, when gum formation occurred further below the surface, discoloration was more extensive than in July or April, when gum formation occurred closer to the surface (Table 12). It was not possible to distinguish after 120 days, those cells which had died with the disorganisation and aggregation of their starch granules in situ, and those cells which had lost their starch by hydrolysis before death. Gum was present in the zone of discolored cells and in the sapwood-discolored wood transition zone. The extent of death of the pith cells was usually less than had occurred in the xylem.

The lack of reaccumulation of starch into pith, xylem and ray parenchyma was a prominent feature of the sapwood-discolored wood transition zone. This was particularly noticeable in December when there was a large accumulation of starch between wounding and harvesting (Table 13). Outside the zone of cells influenced by wounding,
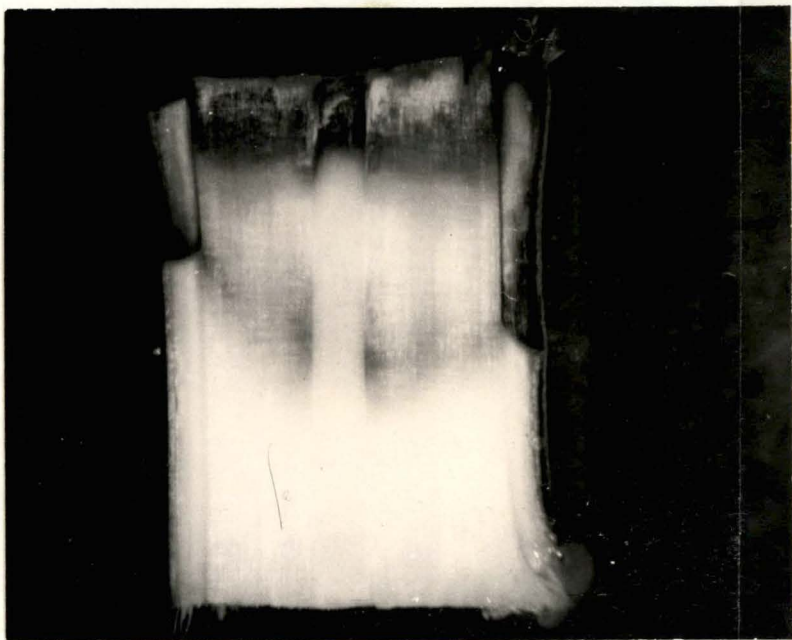
PLATE 37   Distribution and extent of discoloration under a 5-year-old uninoculated STP branch stub, 120 days after wounding. Note the irregular coloration and the darker zone between healthy and discolored wood. The bark of the stub showed physiological dieback with dead and discolored bark extending several mm. downwards from the wound surface. (Wounded December 1969, x 5.)

PLATE 38   Starch grains present in ray and xylem
parenchyma cells at the surface of a
120 day old wound in 5-year-old STP
wood. In the ray and xylem parenchyma
cells beneath the surface zone of starch
filled cells, the starch grains have
become aggregated and disorganised.
(Wounded July 1969, x 200.)

starch accumulated abundantly and the transition in the pith and rays from cells which contained little starch to cells completely filled with starch, occurred over a small number of cells (Plate 39). This effect suggests some type of hormonal influence in wound zones.

Gum formation at T120 was extensive, with occlusion of the vessels over much longer distances than at T15 or T30. The length of the gum zone was significantly greater under wounds made in December than in July or April, and in addition the length of gum formed under July wounds was significantly greater than that under April wounds (Table 12). The longitudinal length of the gum zone tended to be fairly even across the xylem, even though the depth below the surfaces at which it started, varied with age of the xylem. In wounds made in July, the initial gum formed appeared to be mainly small globules, but gum formed in the Spring in these wounds, took the form of larger globules and plugs. This change in morphology was probably related to the marked alteration in physiological activity of the tree, which occurred in the latter part of the 120 day wound period. In wounds made in December and April, gum consisted of a mixture of larger plugs and globules.

Another feature noted on wounds made in December, was the collapse and death of the bark for a variable distance below the wound surface (Plate 37). This symptom differed from the papery bark symptom and occurred only on wounds made in December (mean extent 0.80 cm.). From observation, it appeared that bark death was associated with the more extensive death and discoloration of the wood which occurred under wounds made at that time. This symptom will be referred to as physiological dieback.
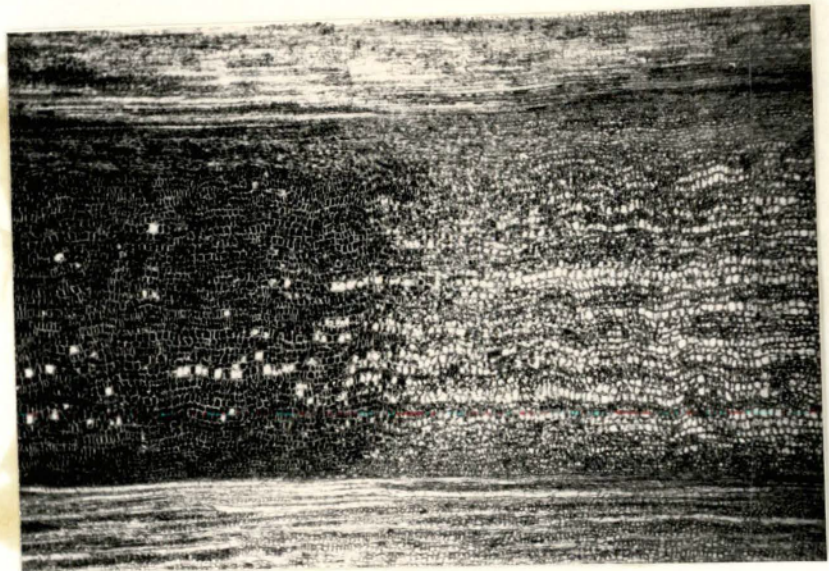
PLATE 39   An example of the accumulation of starch in the pith
            (centre) and xylem (left and right) observed in the
            wood beneath wounds and inoculations made in
            December 1969 and harvested in April 1970. The
            starch concentration per cell decreased rapidly on
            moving from the healthy wood to wood in which
            physiological changes occurred as a result of
            wounding. (TO inoculation, wounded December 1969
            x 32.)

The time from wounding to the initiation of gum formation in the vessels seemed to be little affected by season. After the initiation of gum formation, the rate of gum formation was greater under wounds made in December than in July or April, as evidenced by the greater length of gum formed under December wounds.

While it was not possible to relate directly nutritional status and gum formation, it appeared that the nutritional status of the wood, as well as the increased metabolic activity of the cells, probably favoured gum formation under December wounds. From observation and analyses (Table 13) starch levels in the wood showed a marked increase in December together with smaller rises in the levels of soluble sugars and hemicelluloses. If reserve carbohydrates were the raw materials for gum synthesis, then gum formation would presumably be favoured by the increased starch levels during the summer period. While starch levels were high in April and July the lower rate of cell metabolic activity possibly limited the response. Wood nitrogen and phosphorus levels also showed a seasonal pattern of variation but were lowest during the summer-autumn period.

Season therefore had an effect on the discoloration and gum formation under wounds in young apple wood in four main respects.

(i) The extent of discoloration beneath summer wounds was greater than under wounds made in autumn and winter.

(ii) The depth below wound surface at which gum formation commenced was greater in wounds made in summer than in wounds made in autumn or winter.

TABLE 13

Mean levels of nitrogen, phosphorus and carbohydrates in the 5-year-wood of 40-50-year-old STP trees during 1969-1970. (Series II trees.)

Date of sampling	15/7/69	10/10/69	21/11/69	2/1/70	1/4/70	14/5/70
Nitrogen (% dry matter)	0.284*	0.306	-	0.124	0.121	0.192
Phosphorus (ppm dry matter)	360.0*	302.0	-	253.0	268.0	357.0
Soluble sugars (mg/g dry matter)	16.36[+]	14.13	10.62	11.70	11.69	14.46
Starch (mg/g dry matter)	26.21[+]	15.34	20.97	37.94	40.63	44.27
Hemicellulose (mg/g dry matter)	245.86[+]	232.75	251.10	291.11	285.03	274.92

* N and P levels at each time mean of samples from 18 trees.

[+] Soluble sugars, starch and hemicellulose mean levels for 5 trees at each time (same trees sampled at each time, selected at random from the original 18 trees of Series II).

(iii) The quantity of gum formed after summer wounding was greater than that after wounding in autumn or winter.

(iv) While not measurable factors, the morphology of gum deposits and intensity of gum formation varied between seasons. Gum formed during periods of high physiological activity took the form of large globules or plugs blocking vessels over long distances. Gum formed in the dormant period consisted mainly of small irregular globules, with only partial occlusion of the vessels.

3. The effect of inoculation with T. versicolor, method of inoculation, and season on gum formation under inoculated wounds of different ages, and susceptibility of wounds to fungal invasion.

The experiment was initially designed to examine variations in the seasonal susceptibility of trees to T. versicolor, suggested by the results of Darbyshire (1967). That author used a long term method - up to 12 months following inoculation, before measurement of symptoms. Over such a long period trees would show a whole range of physiological conditions, and it was proposed to examine seasonal susceptibility over shorter time periods, in which physiological changes would be more restricted. Differences in susceptibility between season were measured by external symptoms (papery bark), and by the macroscopic measurement of the length of fungal penetration in the wood. The results as measured by external symptoms, were not as clear as expected. The investigation was therefore extended to a more detailed microscopic examination of the extent of fungal penetration and gum formation,

and the short term relation between the two, in inoculations in young wood. The experimental design was not completely satisfactory for this purpose, lacking suitable controls to examine the effect of method of inoculation on gum formation, but information on a number of aspects was obtained from the experiment.

(a) Extent of external symptoms.

The only papery bark symptoms recorded over the period of inoculation in any season, were on Time 0 inoculations made in July. Only 9 of the 18 replications showed any symptoms and the average extent was 0.7 cm. In addition, wounds of all ages inoculated in December, showed physiological dieback (page 113). The mean length of this symptom was less than 1 cm. Thus, physiological dieback occurred on inoculated and uninoculated wounds.

(b) Extent of fungal penetration.

The mean lengths of hyphal penetration in inoculations and the relevant orthogonal comparisons are shown in Table 14. Both the season of inoculation and the age of wound inoculated, had a highly significant effect on fungal penetration. The greatest penetration occurred in inoculations made in December and the length of penetration was significantly greater than that which occurred in July or April. Significantly greater penetration occurred in July than in April. Plate 40 illustrates the pattern of discoloration and decay under inoculated wounds. Orthogonal comparisons between the ages of wound inoculated, showed the length of penetration that occurred in inoculations at T0 was significantly

TABLE 14

Mean lengths of hyphal penetration and depth below wound
surface at which gum formation commenced in wounds of three
ages inoculated in three seasons. [Each figure represents
mean of 18 observations, analyses of variance Appendix I (e).]

Month of wounding	JULY (J)			DECEMBER (D)			APRIL (A)		
Age of wound inoculated (days)	0	15.	30	0	15	30	0	15	30
Mean length of fungal penetration (mm.)	6.3	3.1	3.2	8.7	4.7	2.8	3.6	1.7	1.9
Mean width of clear zone (mm.)	2.2	1.0	0.0	3.9	3.4	1.6	2.6	2.5	0.0
Total depth below surface gum starts (mm.)	8.5	4.2	3.2	12.6	8.1	4.4	6.1	4.1	1.9

Orthogonal comparisons

	Length of hyphal penetration	Depth below wound surface gum starts
1. Effect of season		
D vs J, A.	***	***
J vs. A.	***	**
2. Effect of age of wound		
0 vs 15, 30	***	***
15 vs 30	n.s.	***
3. Season x age interaction		
D vs J, A at 0 vs 15, 30	***	**
D vs J, A at 15 vs 30	**	*
J vs A at 0 vs 15, 30	n.s.	n.s.
J vs A at 15 vs 30	n.s.	n.s.
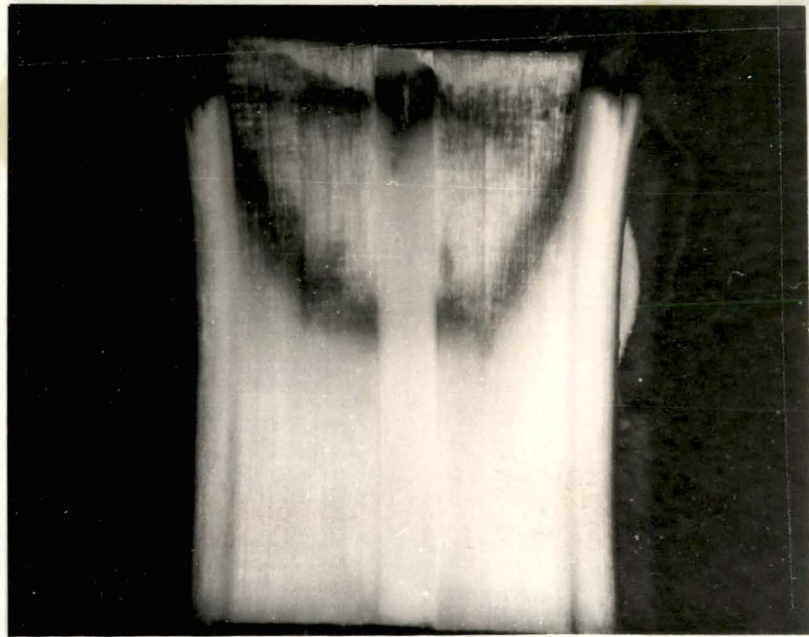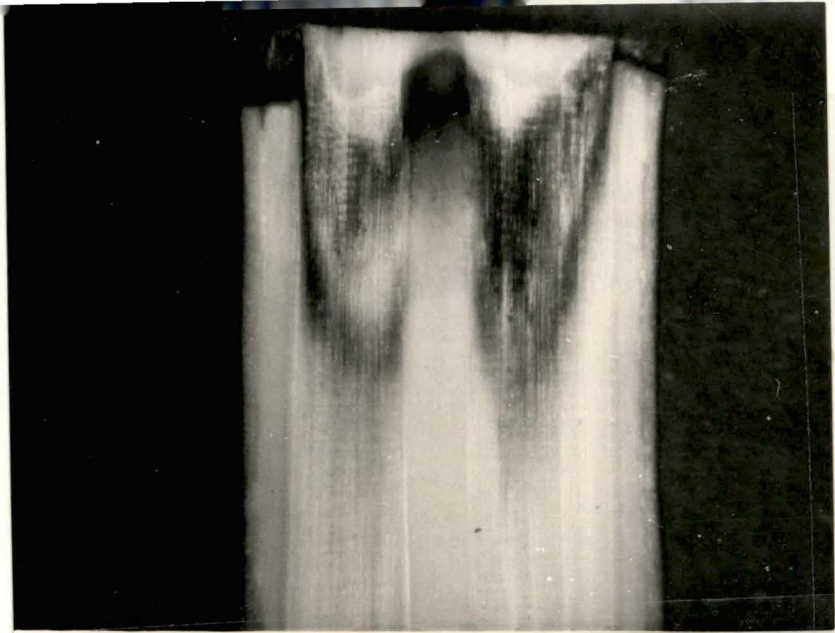
PLATE 40   The effect of ageing of wounds in 5-year-old STP wood
            on their susceptibility to fungal penetration and
            wood decay by T. versicolor. Wounds were made in
            December, 1969 and inoculated at T0 (top) and T30
            (bottom). In the wound inoculated at T0 there was a
            well defined zone of white rot extending downwards
            from the wound surface, but in the wound at T30 there
            was no obvious white rot. The only indication of
            fungal activity in the T30 inoculation was a zone of
            lighter discoloration extending a few mm. immediately
            beneath the wound surface. x 5.

greater than that in wounds inoculated at 15 and 30 days of age, respectively. The analysis of the inter-action between season and age of wound inoculated indicated the effect of age of wound in limiting penetration, was greater during the season of high physiological activity than at other times of the year.

In all seasons, ageing of wounds reduced the amount of fungal hyphae in the wood as well as decreasing the length of fungal penetration. Hyphae were very sparse in inoculations at T15 and T30, while at T0 they were abundant. Isolations showed that the fungus was viable in all ages of wound inoculated at the final harvest (120 days after inoculation) and in some check inoculations after 12 months. Thus, the ability of the fungus to penetrate and proliferate was much reduced in wounds allowed to age for a time prior to inoculation. The greatest penetration occurred when the reserve carbohydrates of the wood were at their highest level (Table 13).

(c) Depth below wound surface at which gum formed.

The mean figures for the depth below inoculations at which gum formation commenced and the relevant orthogonal comparisons are shown in Table 14. It will be seen that the depth below the inoculated surface at which gum formation commenced was the sum of two components – (i) a depth equivalent to the extent of fungal penetration, and (ii) a zone of tissue free of gum and fungal hyphae. In the upper portion of this latter zone, the cells were dead in advance of fungal penetration but in the lower portion the cells, although mostly devoid of starch, appeared to be alive. This zone will be referred to as the clear zone and it graded into the zone of tissue in which gum formation occurred.

It appeared that in these inoculations, insufficient time had elapsed for the fungus to penetrate to the depth of the gum zone, except in the case of T30 inoculations in July and April, when gum formation had already commenced close to the wound surface at the time of inoculation. Checks on inoculations in different trees, showed that this lag phase in establishing the normal relationship between host and fungus, where discoloration and gum formation continually occurred immediately in advance of the fungus as long as the fungus was active (Figure 4), was a consistent feature in newly inoculated young wood.

Both season and age of wound had a significant effect on the depth at which gum formation commenced (Table 14). The depth at which it commenced in December was significantly greater than in July or April and the depth of commencement in July was significantly greater than that in April. Significant differences also existed for this parameter between inoculations at T0 and at T15, T30 and between T15 and T30. The general seasonal effect was the same as that previously described in uninoculated wounds (Table 12).

Variations within seasons of the depth of gum formation, indicated that either inoculation with T. versicolor or the method of inoculation, involving the covering of the stub with aluminium foil for one month following inoculation, increased the distance below the wound surface at which gum formation commenced. It did not seem likely that the fungus alone would affect the depth of gum formation, but it was plausible that covering with aluminium foil could have such an effect. Covered but uninoculated controls were not incorporated in the experimental design and it was not strictly correct to make

comparisons with the uncovered wounds (Table 12). However, the circumstantial evidence that covering did increase the depth of gum formation was strong. Firstly, the depth at which gum formation commenced in wounds inoculated at 15 or 30 days after wounding (Table 14), was similar to that in uninoculated wounds at T30 or T120 (Table 12). Secondly, by T30 a definite gum zone was present under the wound, but in T30 inoculations the inoculation process appeared to have affected gum formation. A visibly less intense zone of gum formation occurred between gum formed by T30, and the normal gum zone which presumably formed after the foil covering was removed. In addition, a small pilot trial to examine the effect of covering on wounds inoculated and uninoculated at T0, showed very little gum formed during the period the wounds were covered (30 days after wounding), in either the inoculated or uninoculated wounds. Most gum formed after removal of the covers and the depth at which it formed was greater than in uncovered controls. Thus it appeared that the method of inoculation as well as the season influenced the depth below the surface at which gum formed in cut stubs.

(d) The extent of gum formation under inoculations.

The results are shown in Table 15. Plate 41 shows the relationship between the extent of discoloration and gum formation, the form of the gum zone and its typical extent.

The results closely parallel those found under uninoculated wounds (Table 12). The length of gum formed was significantly greater in December than in July or April. It was not possible to assess the significance of the differences in length of gum formation beneath wounds of different age, within each season.
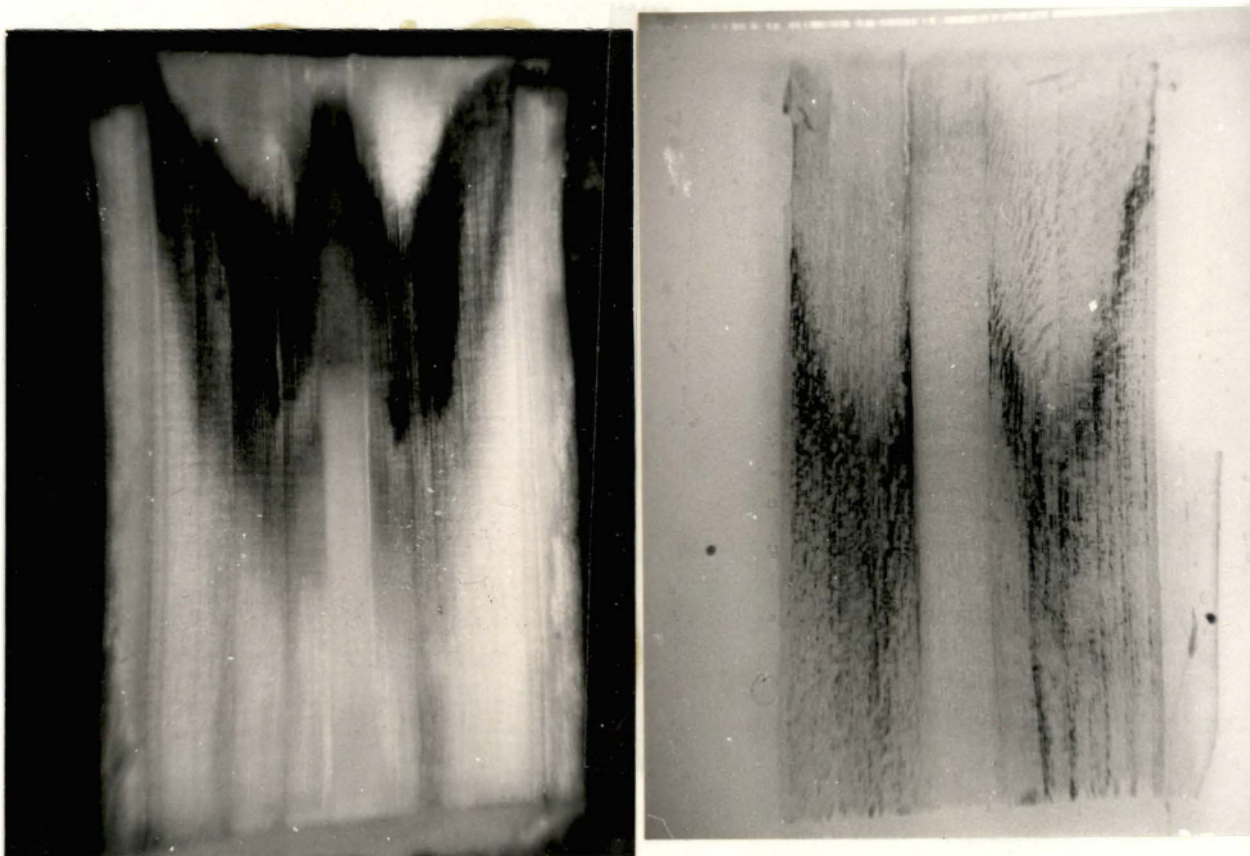
PLATE 41   Illustrating the relationship between fungal penetration,
            discoloration and gum formation in a TO inoculation after
            120 days. The approximate zone of fungal penetration can
            be seen (left) as a light zone of white rot extending
            downwards from the wound surface. The zone of fungal
            penetration was surrounded by a zone of dark discoloration,
            which in turn graded into the sapwood-discolored wood
            transition zone. On the right is an orcinol stained,
            longitudinal section cut from the same stub, which shows
            the gum and its distribution. (Inoculated December
            1969, x 5)

The mean length of gum formation under inoculated wounds within each season (Table 15) was greater than that found under uninoculated wounds in the same season (also shown in Table 15). This suggests that either the method of inoculation or T. versicolor had a stimulating effect on gum formation but evidence favoured the former. It appeared unlikely that T. versicolor had a stimulatory effect on the amount of gum formation as in most inoculations a zone of clear tissue existed between the maximum hyphal penetration and the zone of gum formation. Also in T15 and T30 inoculations in July and April, where the fungus penetrated to the gum zone, length of gum formed was similar to the mean length of gum formed for uninoculated controls.

With regard to the establishment of inoculations of T. versicolor in young apple wood the experiment showed -

(i) The method of inoculation affected the host reaction in at least two ways. Firstly, it prevented gum formation during the period the stubs were covered with aluminium foil following inoculations and secondly, it probably increased the depth below the wound surface at which gum formation would have occurred naturally.

The changes induced by the method of inoculation were superimposed on variations in host reaction due to season (depth and extent of gum formation). Thus, following inoculation, there was a delay in establishing the normal relationship between host and pathogen while the fungus penetrated to the gum zone, whose initial depth of formation and extent were controlled by season and method of inoculation.

(ii) In the short term at least, there seemed little evidence that the fungus stimulated gum formation.

TABLE 15

Mean length of gum formed beneath inoculations 120 days old
on wounds of three ages in three seasons of inoculation.
[Each figure repeesents mean of 18 observations, analysis
of variance Appendix I(f).]

Month of wounding	JULY (J)			DECEMBER (D)			APRIL (A)		
Age of wound inoculated (days)	0	15	30	0	15	30	0	15	30
Mean length of gum formation (mm.)	7.6	4.3	5.1	13.8	11.7	13.5	5.2	4.2	5.0
Mean length of gum under inoculation within each season (120 days old) (mm.)		5.7			13.0			4.8	
Mean length of gum in uninoculated wounds (120 days old) (mm.)		5.1			9.5			3.3	

Orthogonal comparisons

    1. Effect of season

        D vs J & A   ***

        J vs A n.s.

    2. Effect of age of wound

        - comparisons not valid.

(iii)   Fungal penetration was greater from summer inoculations than from winter or autumn inoculations.

(iv)   The changes that occurred in the wood as a result of wounding, apparently led to a rapid decrease in the susceptibility of wounds to fungal invasion - both in terms of the length of hyphal penetration and the amount of hyphae.

SECTION III

External factors affecting the susceptibility of apple trees to _Trametes_ _versicolor_.

INTRODUCTION

Little is known of the factors that influence the susceptibility of apple trees to _Trametes versicolor_. Wade (1968) proposed that mineral nutrition may influence susceptibility and showed phosphorus deficiency increased the susceptibility of young apple trees to the disease. The physiological explanation of this effect is unknown but it may be related to the hypothesis that attack by _T. versicolor_ is favoured by low carbohydrate levels in the wood (Darbyshire, 1967). Phosphorus nutrition was therefore selected for further study in an attempt to explain its effect on susceptibility.

Under Tasmanian field conditions two major factors which influence tree growth and fruiting, and thought likely to be of significance to _T. versicolor_ attack, are nitrogen nutrition and soil moisture status. While both water logging and water stress may be seasonal problems in Tasmania, due to difficulties in controlling the former experimentally, only water stress was considered in this study. As well as its essential role in tree nutrition, nitrogen has frequently been shown to influence susceptibility of wood to decay, _in vitro_.

Two experiments were undertaken to test the effects of these factors as they affected the susceptibility of young trees directly, and in relation to their effects on the carbohydrate status of the wood. Nitrogen nutrition and water stress were grouped in one experiment because of the relatively rapid and direct effects of these two factors on tree physiology. A separate experiment was undertaken on phosphorus nutrition because of the time required to deplete wood phosphorus reserves in established experimental trees.

MATERIALS AND METHODS

(A) Trees, containers, growth media, general cultural practices.

1. Nitrogen and water stress (N x W) experiment.

In August 1968, ninety 3-year-old Sturmer Pippen (STP) trees on seedling rootstocks were planted into 40 litre plastic buckets in a medium composed of 50% fine sand and 50% peat moss. The sand-peat mixture was chosen because of its good physical characteristics and greater water holding capacity over a wider range of moisture tensions than pure sand (Boggie, 1970). Free drainage from buckets was ensured by placing 3-5 cm. of coarse blue metal in the bottom of each bucket. The containers were fitted with plastic lids to minimise evaporation and the entry of rain water.

2. Phosphorus (P) experiment.

In September 1967, ninety 1-year-old STP trees on seedling root-stocks were planted in coarse sand in 100 litre tarred steel drums. A 5 cm. layer of coarse blue metal was added to the drums prior to planting to facilitate drainage and aeration and the sand surface was left uncovered.

3. Trees used for wounding and inoculation studies in Sections I and II.

Trees were 3- and 5-year-old STP trees grown in sand culture as in the P experiment. Trees received complete nutrient solution (Table 16).

All trees were grown outdoors and deflowered after full bloom each season. Insects and fungal diseases were controlled by spraying. Dead leaf material which lodged on the container surfaces was removed to prevent recycling of nutrients.

(B) Experimental designs and treatments.

1. N x W experiment.

A 3 x 2 factorial design was used with three nitrogen treatments [deficiency (N_0), normal (N_1), high (N_2)] and two watering regimes (W_0, W_1). At the commencement of the experiment (August 1968), trees were grouped into fifteen blocks, each of six trees, on the basis of trunk girth and shoot growth in the previous season. The six treatments were allocated randomly within each block, and treatments were applied for the 1968-69 and 1969-70 growing seasons.

Nitrogen treatments were supplied as nutrient solutions containing the required nitrogen level. Nutrient solutions were based on the standard Long Ashton solutions (Hewitt, 1966) and are shown in Table 16. All solutions were made up with tap water [analysis Appendix 2(a)]. Nutrient solutions were applied to each tree at the rate of two litres, twice per week from the October 1 until April 30 each season. All containers were flushed every six weeks with tap water to remove excess salts.

Watering regimes were as follows: W_1 trees were watered every 1-2 days to container capacity (White and Mastalerz, 1966) with nutrients or additional water. Container capacity was determined for each pot at

the beginning of each season as the weight per pot after saturating with water and allowing to drain overnight. Nutrients and water were added to a constant weight. W_0 trees were allowed to wilt before rewatering to container capacity. The weights of the containers at wilting point were recorded and this allowed rewatering with minimal drainage. Pot weights were approximately 27.5 kg. at container capacity and 20 kg. at wilting point. It was necessary to treat W_0 treatments as for the W_1 treatments for the first 4 to 5 weeks of growth each season until tree water usage was sufficient to deplete the available moisture in the containers fairly rapidly. From mid-November to April, depending on weather conditions, the N_1W_0 and N_2W_0 trees reached wilting point in approximately 6 days and N_0W_0 trees in approximately 14 days. Nutrients and water were added so that all treatments received approximately the same total amount of nutrients each season.

Throughout each winter the tops of the containers were covered with plastic sheeting and the trees stored outdoors at container capacity for the W_1 treatments and approximately half container capacity for the W_0 treatments.

2. P experiment.

For the season following planting (1967-68) the trees were maintained on a complete nutrient solution (Wade, 1968) supplied at the rate of 4 litres per week. In August 1968, the trees were grouped into 30 blocks of three trees on the basis of trunk girth and shoot growth the previous season. Within each block, three phosphorus treatments [deficiency (P_0), normal (P_1), high (P_2)] were allocated at random.

TABLE 16

Composition of nutrient solutions used in the nitrogen
and water stress experiment (figures in g/l).

Macronutrients	N_0 (N = 0 ppm)	N_1 (N = 120 ppm)	N_2 (N = 300 ppm)
KNO_3	–	0.404	0.404
$Ca(NO_3)_2.4H_2O$	–	0.360	0.360
$MgSO_4.7H_2O$	0.368	0.368	0.368
KH_2PO_4	0.136	0.136	0.136
$CaCl_2.2H_2O$	0.588	0.368	0.368
NH_2CONH_2	–	0.043	0.386
K_2SO_4	0.348	–	–
Micronutrients			
H_3BO_3	0.00275		
$CuSO_4.5H_2O$	0.0010		
$ZnSO_4.7H_2O$	0.0010	As for N_0	As for N_0
$MnSO_4.4H_2O$	0.00275		
$NH_4Mo_2O_7$	0.0010		
$FeCl_3$	0.0180		

The five blocks at each end of the experimental layout were treated as buffer rows. Nutrient solutions of the following composition were supplied at a rate of two litres, twice per week from October 1 until April 30 each season: (a) minus phosphorus (P_0) - KNO_3 (0.404 g/l), $Ca(NO_3)_2.4H_2O$ (0.360 g/l), $MgSO_4.7H_2O$ (0.368 g/l), $CaCl_2.2H_2O$ (0.368 g/l), NH_2CONH_2(0.043 g/l), KCl (0.0745 g/l); (b) normal phosphorus (P_1, P = 31 ppm) - complete nutrient solution as in Table 16; (c) high phosphorus (P_2, P = 93 ppm) - as for the complete nutrient solution plus an additional 0.312 g/l of $NaH_2PO_4.2H_2O$. Micronutrients were added to all solutions (Table 16) and solutions were made up with tap water.

The depletion of tree phosphorus reserves to a deficiency level in the P_0 trees was calculated to take two seasons based on estimates of tissue phosphorus content and projected seasonal dry matter production.

Nutrients treatments were applied for the 1968-69, 1969-70 and 1970-71 growing seasons. Additional water was supplied to containers through fine nozzles fitted into a PVC pipe set above each container. This supply also allowed regular flushing of the containers to remove excess salts. During the 1969-70 season some trees showed symptoms of magnesium deficiency and five magnesium sprays (2% $MgSO_4.7H_2O$) were applied during each of the following seasons.

(C) Inoculation with T. versicolor and symptom measurement.

1. N x W experiment.

In July 1969, nine blocks of trees were selected at random from the original fifteen blocks for inoculation with T. versicolor. The remaining six blocks were used for sampling at intervals during the period of inoculation to check tree nutrient status. Each tree had three major scaffold branches and one branch of each tree was selected, cut back to the 3-year-old wood, and inoculated by the method previously described (page 42). The point of inoculation in the 3-year-old wood was selected so that the diameter of the inoculated limbs in all treatments was in the range 1.3-1.4 cm. Buds that grew out around the inoculated stub the following growing season were rubbed off, leaving approximately 6 cm. of bare wood to the first active node.

2. P experiment.

Twenty replications were used for the experiment and twelve of these were selected at random in July 1970, for inoculation. The eight remaining replications were harvested at four times (2 replications per harvest) during the period of inoculation to check nutrient status of trees. Two limbs per tree were cut back to the 2-year-old wood (diameter 1.3-1.4 cm.) and inoculated. No attempt was made to discourage the outgrowth of buds beneath the cut stubs which occurred in the following season. This practice was adopted for the P experiment because in the N x W experiment rubbing off the buds led to the occurrence of a dying back of the inoculated stubs, unrelated to infection by T. versicolor.

In the N x W experiment, the length of the bark symptoms was measured at the final harvest in July 1970 (total ~~inocu~~lation time *incub-* 12 months). The extent was measured at four equally spaced points around the limb using the vertical plane of the limb (if the limb was pulled horizontal) as a reference. From these four measurements a mean length of bark symptoms per stub was calculated. In the P experiment the extent of bark sypmtoms was measured in the same manner three months after inoculation and every month thereafter until the final harvest in June 1971 (total ~~inocu~~lation time 10 months). *incub-*

The extent of internal penetration by T. versicolor was measured by splitting the limbs in longitudinal section at right angles to the axis of the tree. Penetration was measured macroscopically from the point of inoculation to the zone of incipient decay at seven points across each limb (Figure 7). This method of measurement of hyphal penetration was found to give comparable results to microscopic measurements of hyphal penetration on this sections.

Thin sections were cut and stained for fungal hyphae, gum and starch as previously described (pages 43-44).

(D) <u>Growth and water stress measurements, tissue sampling for mineral and carbohydrate analyses.</u>

1. <u>Growth measurements.</u>

The total length of new shoot growth was measured on all trees in each experiment after leaf fall each year. Fifty per cent (length basis) of each new shoot was removed in pruning, and the weight of

FIGURE 7

Diagram indicating the seven points at which measurements were made
of the length of penetration by $\underline{T}$. versicolor in branch stubs in
the N x W and P experiments.

INOCULATION
SURFACE

PAPERY
BARK

INCIPIENT
DECAY ZONE

DISCOLORED
WOOD

BARK

SAPWOOD

PITH
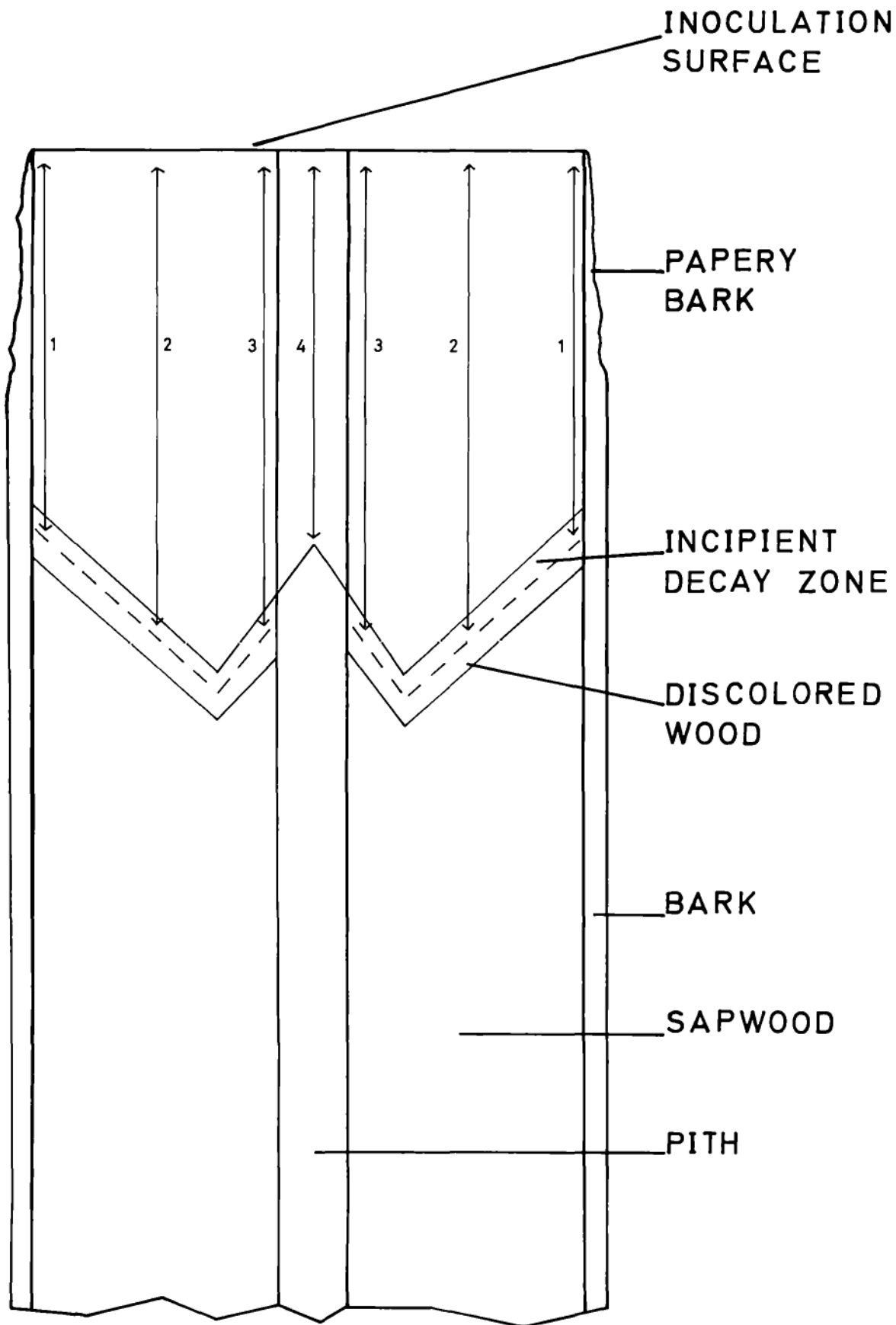
prunings was recorded for each tree. After pruning to this formula it was usually necessary to remove some shoots entirely to control tree form.

2. Leaf water potential measurements.

Leaf water potentials were determined using a pressure chamber of similar design to that described by Klepper and Ceccato (1969). Measurements were made on midshoot leaves selected at random from the main shoots. Readings were made at hourly or two hourly intervals. General weather conditions on the days of measurement were fine and warm.

3. Tissue sampling.

Leaf samples for analyses were taken from trees of each experiment at the end of January each year. The two midshoot leaves from all shoots were collected and bulked to give a single sample of leaves per tree. Leaves were washed in distilled water, oven dried at $65^{\circ}C$ for 48 hours and ground in a Culatti hammer mill. Samples were stored in closed glass vials at $-20^{\circ}C$ until analysis.

Wood of the same age removed from limbs at inoculation was retained from each experiment. The bark and wood were separated and in the N x W experiment oven-dried at $65^{\circ}C$ for 48 hours. For the P experiment freeze-drying facilities were available (Dynavac FD 16 High vacuum freeze drying unit) and tissues were preserved in this manner. After drying or freeze drying, each bark and wood sample was ground in a Wiley Mill No.1 and then in a Culatti hammer mill. The double grinding was found necessary to ensure the uniform mixing of particle sizes in the comminuted material.

The above ground portions of uninoculated trees sampled during periods of inoculation, were separated into current seasons growth and wood of different ages. The latter material was separated into bark and wood. Tissues were dried, ground and stored as described above.

(E) Methods of analysis.

Leaf samples collected in January 1970, from trees in both experiments were analysed for five major elements (N, K, Ca, P and Mg) and three minor elements (Mn, Fe and Cu) by the Tasmanian Government Analyst. All other mitrogen, phosphorus and carbohydrate analyses were carried out by the author.

1. Nitrogen.

Total nitrogen (not including nitrates) content of samples were determined using the micro-Kjeldahl method of Markham (1942). A mixture of K_2SO_4 and $CuSO_4.5H_2O$ (14:1) was used as a catalyst on the recommendation of the Tasmanian Government Analyst.

2. Phosphorus.

This element was determined using the molybdo-vanadate method of Jacob and Hoffman (1954). One-tenth of a gram of material (leaves, bark) or 0.2 g. wood was digested and its phosphorus content determined following the procedure of Lamp (1968). Nitrogen and phosphorus analyses were performed in duplicate on each sample.

3. <u>Other cations.</u>

K, Ca, Mg, Mn, Fe and Cu were determined by Atomic Absorption spectrophotometry on nitric/perchloric acid digests of leaf material.

4. <u>Carbohydrate analyses.</u>

Oven dried or freeze dried tissue samples (1.0 g. wood, 0.4 g. bark) were extracted following the procedure of Priestley (1962a). A single sample of each individual wood or bark sample was extracted. Each wood or bark sample gave three extracts which contained soluble sugars and glycosides, starch and hemicelluloses respectively. Sugar concentrations in the extracts were assayed as follows:

(a) Soluble sugars

The soluble sugar extracts from wood and bark were concentrated to 25 ml. One ml. aliquots of the extracts were partially purified on ion exchange resin columns following the methods of Splittstoesser (1969). Glass columns (8 mm. I.D.) were filled to a depth of 5 cm. with Dowex 1 (x4 50/100 mesh, formate form), a piece of nylon fibre inserted into the tube and a 5 cm. depth of Zeocarb 225 (H^+ form) added to the column. The extracts, applied to the columns, were washed through with distilled water to a volume of 50 ml. The treatment resulted in clear to slightly turbid solutions suitable for sugar assay. The resins in the columns were renewed after three solutions had passed through each one. Passing solutions through resin columns removed acidic and basic substances including amino acids, organic acids and glycosides.

Extracts from the columns were assayed by the phenol-sulphuric acid method of Dubois <u>et al.</u> (1956). One ml. of the extract (containing 20-60 µg sugar) was added to a dry dust free test tube.

One ml. of 5% phenol in water was added followed by 5 ml. of concentrated H_2SO_4. Solutions were mixed and allowed to cool in a water bath at $30^{\circ}C$ for 20 minutes. The absorbance was then measured at 490 mμ on a Hitachi Perkin-Elmer UV-VIS Spectrophotometer. Glucose was used as a standard over a range of 0-70 μg and each solution was assayed in duplicate.

(b) Starch.

The hot water extract from wood or bark was brought to 200 ml. with distilled water. Wood extracts required five-fold dilution to achieve a suitable range for assay but the bark extracts could be assayed directly.

The extracts were assayed in duplicate using the phenol-sulphuric acid method of Dubois et al. (1956) or anthrone. The anthrone method was adopted from procedures used by Haas and Fleischman (1958), Hassid and Neufield (1964) and de Bruyn et al. (1969). Both methods gave equally satisfactory results.

Anthrone reagent was prepared by dissolving anthrone (100 mg./ 100 ml.) in 76% H_2SO_4. After the anthrone had dissolved, the solution was heated in a vigorously boiling water bath for 15 minutes and then quickly cooled. The solution was kept in a refrigerator after cooling and until used (with 2-3 hours of preparation). The reagent was prepared daily.

For assay, 2 ml. of the sugar solution was added to a dry-dust-free test tube and 10 ml. of the anthrone reagent added. After mixing the tubes were placed in a boiling water bath for 8 minutes for full development of color. The tubes were cooled quickly and after 30 minutes the absorbance was determined at 625 mμ on a Hitachi Perkin-

Elmer UV-VIS Spectrophotometer. Duplicate assays were undertaken on each solution. Glucose was used as a standard and for starch extracts and calculations were multiplied by 0.9 to correct for hydrolysis.

(c) Hemicelluloses.

Hemicelluloses were determined using a modified anthrone procedure. Bailey (1958) showed pentose sugars formed a stable color with anthrone in proportion to the sugar concentration, provided no excess anthrone was present. Excess anthrone (e.g. as used for hexose assay) destroyed the color, leading to serious underestimation of sugar content.

Anthrone reagent was prepared as above, but using an anthrone concentration of 10 mg./100 ml. of 76% H_2SO_4. The assay was carried out by the procedure used for hexoses. The acid hydrolysis extracts from wood (40 ml.) were filtered and made to 100 ml. with distilled water. This solution was diluted fifty-fold and duplicate 2 ml. aliquots of the diluted solution assayed for sugars.

Apple hemicelluloses consist mainly of long chains of xylose units (Dutton and Murata, 1961). D-xylose was therefore used as a standard over a range of 0-150 μg and the hemicellulose content expressed in terms of xylose equivalents. The method was found to give equivalent results to the iodometric assay method employed by Priestley (1962a).

(F) Statistical analyses.

Analyses of variance were conducted in the normal manner. Main treatment effects were partitioned into single degree of freedom orthogonal comparisons to separate statistically significant effects (Steel and Torrie, 1960).

OBSERVATIONS AND RESULTS

(A) Effect of nitrogen status and water stress on the susceptibility of young STP trees to T. versicolor.

1. Effects of treatments on tree growth and nutritional status.

(a) Leaf symptoms and growth effects.

Nitrogen and water treatments were applied to the trees for the season prior to inoculation (1968-69) and for the growing season during the period of inoculation (1969-70). The effects of nitrogen deficiency were obvious by late November during the first season, and gradually increased in severity over the remainder of the experimental period. The observed symptoms of nitrogen deficiency were similar to those described by Wallace (1951) and included greatly reduced shoot growth (both in terms of growth per shoot and in numbers of shoots per tree) and a general reduction in secondary growth. Leaves were smaller in size and a yellowish-green. Nitrogen deficient trees defoliated earlier than trees of the normal and high nitrogen treatments. Visually, there appeared little difference between trees on normal and high nitrogen treatments. Trees on the plus nitrogen treatments were very vigorous with plentiful shoot growth and dark green leaves. Plate 42a and b illustrates differences between trees of the six treatments during the second season.

The effects of water stress on growth, were noticeable during the first season but were more marked in the second season. The major effects of water stress were a reduction in the length of shoots, although

PLATE 42   (a) Top: trees of W_1 treatment.   From left
to right – N_2, N_1, N_0.
(b) Bottom: trees of W_0 treatment.   From
left to right – N_2, N_1, N_0.

Note differences in growth between trees of
W_0 and W_1 treatments and between trees having
the same water treatment but supplied with
different levels of nitrogen.   The leaves of
the N_0 trees appeared yellowish-green
compared with leaves of N_1 and N_2 trees.
Trees of N_1W_0 and N_0W_0 treatments show
wilting symptoms.   Inoculated stubs can be
seen on trees in treatments N_0W_0 and N_1W_0.
(Photographed February 1970.)

this was compensated for by some thickening of the shoots in the N_1 and N_2 treatments, and a reduction in the number of shoots per tree compared with treatments watered normally (compare Plate 42 a and b). Water stressed trees defoliated earlier compared with trees receiving the normal watering treatment.

During the second season the magnitude of the internal water stress in W_0 trees was examined and compared with that in W_1 trees. The diurnal fluctuations in internal water stress in the trees of W_1 treatments and the W_0 treatments immediately after rewatering were found to be similar to those described for pears (Klepper, 1968). The lowest water potentials were recorded at 1200-1400 hours and were normally of a magnitude of -15 to -20 bars (Figure 8). The stress in the N_1 and N_2 treatments was generally somewhat greater than that in the N_0 treatments. During the drying period in the W_0 treatments, the average maximum daily stress increased, although prior to the development of wilting symptoms it returned to a low level at night (Figure 8). During the day that the trees developed visible wilting symptoms, the maximum daytime stress increased markedly. The minimum water potential decreased from the normal -15 to -20 bars to -25 to -32 bars, and although the potential increased slightly after the minimum at 1200-1400 hours, it continued at an abnormally low level. Rewatering at this stage allowed the trees to regain turgor over-night. An example of the changing pattern of stress is shown in Figure 9 where stresses are plotted as the magnitude of the differences in leaf water potentials in trees of W_0 and W_1 treatments at the N_1 level. The effect of withholding water from trees, led to progressively greater maximum daily water stresses (higher stresses for longer times) over

FIGURE 8

Pattern of diurnal fluctuation in leaf water potential observed in trees of W_0 and W_1 treatments on March 13, 1970. W_1 trees were watered the day measurements were made, while W_0 trees had been rewatered following wilting, on March 10, 1970. Similar diurnal fluctuations in water stress occurred in W_0 and W_1 trees after watering but with utilisation of the available soil moisture, maximum daytime stresses increased in the W_0 trees. On the day of measurements the minimum daytime leaf water potential was 4-8 bars lower in W_0 trees (below broken line) than in W_1 trees (above broken line).

TIME OF DAY (HOURS)
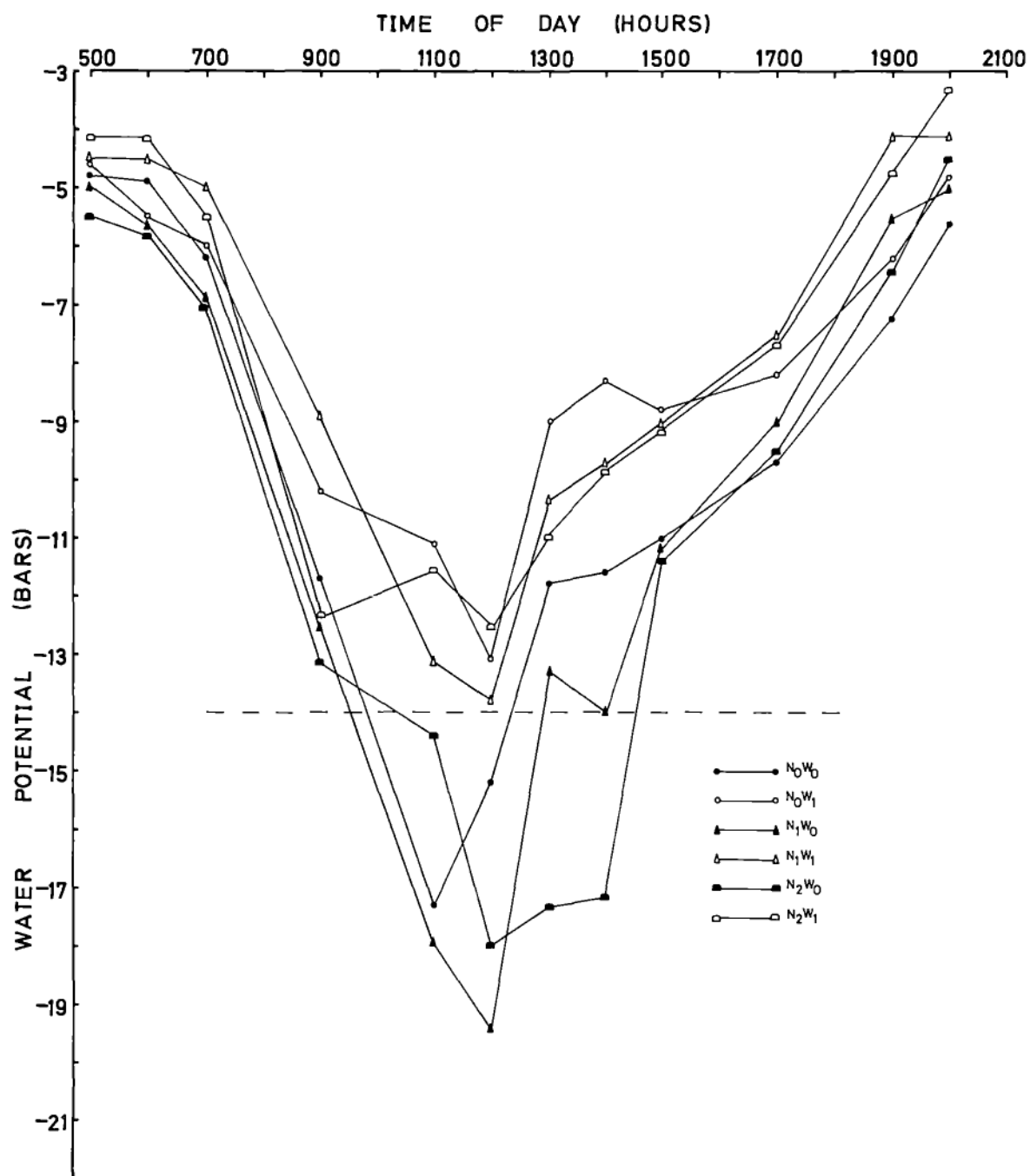
FIGURE 9

The magnitude of the differences in water potential
(measured in mid-shoot leaves) between a tree of the N_1W_0
treatment and a tree of the N_1W_1 treatment, at four times
of measurement over the drying cycle. The level of water
stress in the N_1W_0 tree gradually increased over the six
day period and as the tree developed wilting symptoms
(March 16, 1970), leaf water potential dropped to a very
low level.
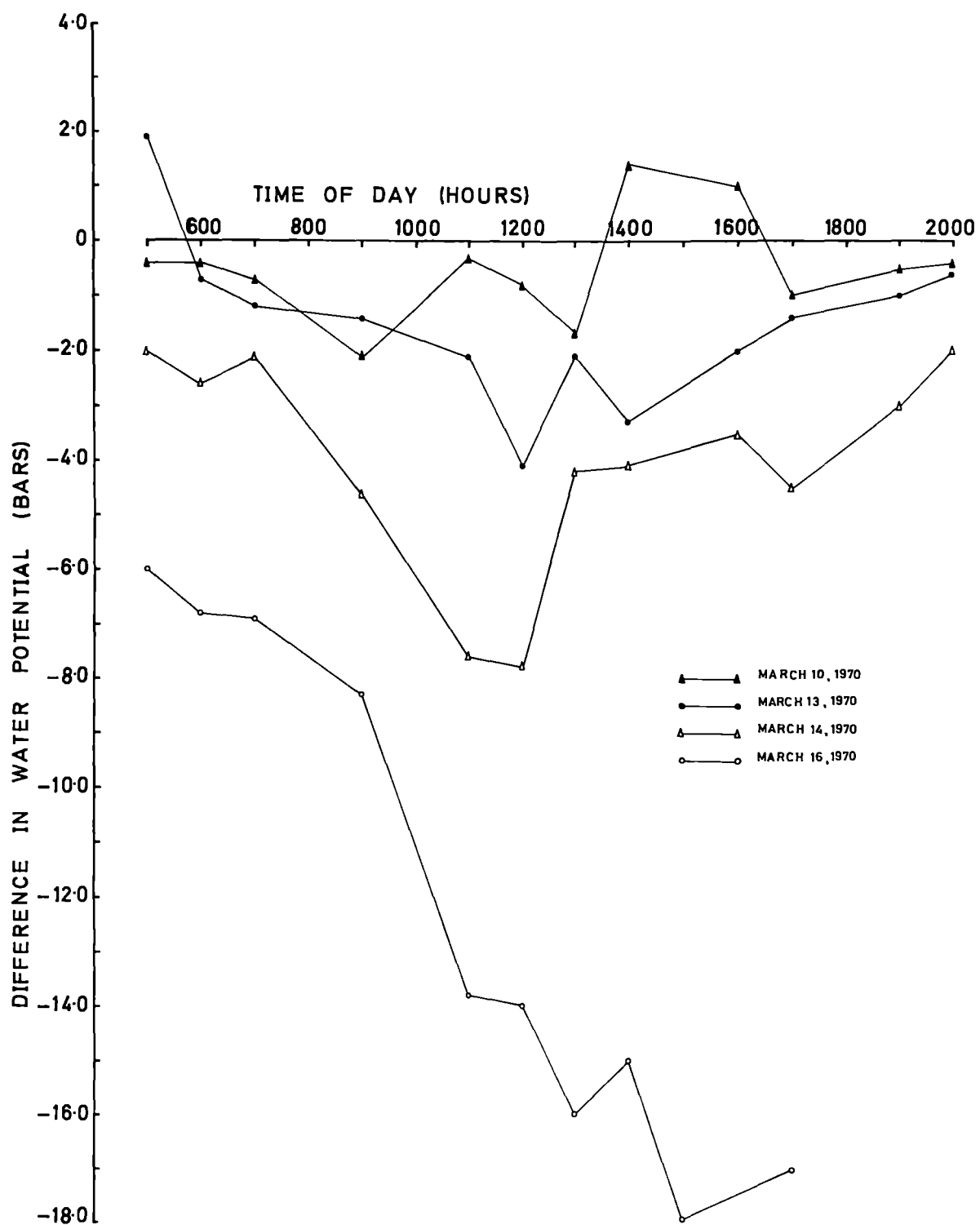
the drying cycle, with the development of very low water potentials at wilting.

The effect of the nitrogen and watering treatments on tree growth was examined quantitatively by shoot growth measurements and by measurements of the weight of prunings. The means and orthogonal comparisons are shown in Table 17. Data on which Table 17 is based is given in Appendix 2(b). In general, treatment effects on growth were more severe in 1969-70 than in the first season of treatment. This was probably a conditioning effect of the treatments on physiological processes and reserve balances which would have been absent at the commencement of treatments. However, differences between W_0 and W_1 treatments and N_0 and N_1, N_2 treatments were highly significant for both parameters in each season. The effects of water stress on tree growth were similar to those reported by Kenworthy (1949). In 1968-69, the high nitrogen treatment appeared to have an inhibitory effect on shoot length compared with normal nitrogen treatment (differences significant at 1% probability). However, the difference between the weight of prunings for N_1 and N_2 treatments was not significant, which indicated the reduction in shoot growth was compensated either by increased shoot diameters or greater shoot numbers. Although precise shoot counts per tree were not made, observations favoured the latter explanation. In 1969-70, shoot growth was greater in N_2 trees than in N_1 trees as was the weight of prunings (significantly different at 1% probability). In 1969-70, there was also a nitrogen and water treatment interaction with water stress producing a greater reduction in shoot growth and weight of prunings at the low nitrogen level than at the higher nitrogen levels. However, the additional nitrogen supply made only small differences to growth between

TABLE 17

The mean lengths of shoot growth and mean weights of prunings
per inoculated tree for trees in the N x W experiment for two growing seasons.[†]

	N_0W_0	N_0W_1	N_1W_0	N_1W_1	N_2W_0	N_2W_1
Mean shoot length/tree 1968-69 (cm.)	306	449	613	830	549	699
Mean weight of prunings per tree 1968-69 (g)	42	67	123	150	129	170
Mean shoot length per tree 1969-70 (cm.)	252	565	776	1524	800	1619
Mean weight of prunings per tree 1969-70 (g)	14	52	78	208	96	244

[†] Each figure is the mean of nine replications.

Orthogonal comparisons

1. Mean shoot length per tree 1968-69
    (a) W_0 vs W_1***
    (b) N_0 vs N_1, N_2***
    (c) N_1 vs N_2**
2. Mean shoot length per tree 1969-70
    (a) W_0 vs W_1***
    (b) N_0 vs N_1, N_2***
    (c) N_1 vs N_2 n.s.
    (d) W_0 vs W_1 at N_0 vs N_1, N_2***
3. Mean weight of prunings per tree 1968-69
    (a) W_0 vs W_1***
    (b) N_0 vs N_1, N_2***
    (c) N_1 vs N_2 n.s.
4. Mean weight of prunings per tree 1969-70
    (a) W_0 vs W_1***
    (b) N_0 vs N_1, N_2***
    (c) N_1 vs N_2**
    (d) W_0 vs W_1 at N_0 vs N_1, N_2***

Asterisks indicate level of significance and apply to other tables in
Section III.

*** = 0.1%   ** = 1.0%   * = 5.0%

the N_1 and N_2 treatments compared with the major effects of nitrogen deficiency and water stress on tree growth.

(b) Nutritional status of the trees at inoculation and during the period of inoculation.

Preliminary analyses of wood and bark for nitrogen, phosphorus and carbohydrates at the end of the 1968-69 season, indicated that differences in the nutritional status of trees due to treatment had occurred. On this basis, it was decided to inoculate trees with $\underline{T}$. versicolor. Levels of nitrogen, phosphorus and carbohydrates determined in wood removed from inoculated limbs at inoculation, were taken to be representative of the nutritional status of the wood inoculated. In addition, wood and bark samples from limbs equivalent to those inoculated but from uninoculated trees, were taken during 1969-70 to check the tree nutritional status. Leaf samples from inoculated trees were also analysed.

(i) Inoculated limbs.

Nitrogen and phosphorus.

The mean levels of nitrogen and phosphorus in inoculated limbs and the orthogonal comparisons are shown in Table 18. Data on which Table 18 is based is given in Appendix 2(c). Nitrogen contents of wood and bark were significantly different at the three nitrogen levels used. The bark nitrogen content was 4-5 times that of the wood.

Nitrogen supply significantly increased the levels of phosphorus in the bark and wood of trees in the N_1 and N_2 treatments compared with N_0 trees (Table 18). The phosphorus concentration in the bark was significantly higher in the N_2 trees compared with N_1 trees. This difference was not apparent in the wood. Yokomizo et al. (1964)

TABLE 18

The mean levels of nitrogen and phosphorus in the wood and bark
of 3-year-old inoculated limbs of trees in the N x W experiment. †

	Nitrogen (% d.m.)		Phosphorus (ppm d.m.)	
Treatment	Bark	Wood	Bark	Wood
N_0W_0	0.98	0.25	1738	500
N_0W_1	1.12	0.26	1588	451
N_1W_0	1.39	0.35	2387	613
N_1W_1	1.35	0.33	2314	602
N_2W_0	1.67	0.42	2815	738
N_2W_1	1.64	0.39	2351	613

† Each figure is the mean of nine replications.

Orthogonal comparisons

		Wood	Bark
1. Nitrogen			
(a) N_0 vs N_1, N_2		***	***
(b) N_1 vs N_2		***	***
(c) W_0 vs W_1		n.s.	n.s.
2. Phosphorus			
(a) N_0 vs N_1, N_2		***	***
(b) N_1 vs N_2		n.s.	*
(c) W_0 vs W_1		*	*

reported increased bark and wood phosphorus levels in 5-year-old Jonathon trees with increasing nitrogen supply.

Water stress led to increased phosphorus levels (differences significant at 5% probability) in the wood and bark of trees in all treatments, a result contrary to the findings of Mason (1958). At the time of inoculation water stress had not significantly influenced the nitrogen content of 3-year-old wood or bark.

Nitrogen and phosphorus status, examined in 1- and 3-year-old wood of uninoculated trees at six sampling times during the period of inoculation, maintained the same general relationship to treatments (Table 19). Water stress affected the nitrogen content of wood and bark more strongly during the second season however. The concentration of nitrogen in the wood and bark tended to be increased in trees subjected to water stress in the N_1 and N_2 treatments but the reverse was true in the N_0 trees. Although the differences were not significant, a similar trend existed in the nitrogen levels of the 3-year-old wood and bark of inoculated limbs (Table 18). Thus, it seemed a period of conditioning was required before the full effects of water stress became evident on the nitrogen status of the trees. Mason (1958) and Mochizuki (1963) reported increases in the nitrogen content of wood, bark and leaves of apple trees subjected to water stress. The reason for the opposite effect in the trees of the N_0 treatment in this experiment is unknown.

Carbohydrate status.

Table 20 shows the mean levels of the three carbohydrate fractions in the 3-year-old wood at inoculation and the orthogonal comparisons. The full data is given in Appendix 2(d). The level of soluble sugars in the N_0 trees was significantly lower than that in the N_1 or N_2 trees,

TABLE 19

The mean nitrogen and phosphorus contents of 1- and 3-year-old wood
and bark of uninoculated trees during 1969-70.†

	Nitrogen (% d.m.)				Phosphorus (ppm d.m.)			
	1-year-wood		3-year-wood		1-year-wood		3-year-wood	
	Bark	Wood	Bark	Wood	Bark	Wood	Bark	Wood
N_0W_0	0.64	0.16	0.58	0.12	1629	444	1748	428
N_0W_1	0.74	0.19	0.69	0.16	1519	418	1503	424
N_1W_0	0.98	0.26	0.93	0.22	2123	554	2254	483
N_1W_1	0.91	0.23	0.86	0.20	1912	453	1980	456
N_2W_0	1.59	0.49	1.42	0.35	3508	954	3149	583
N_2W_1	1.14	0.38	1.03	0.29	2305	617	2118	456

† Each figure is the mean of values for six sampling times during
1969-70. One replication of each treatment was harvested at
each sampling time.

TABLE 20

Mean levels of three carbohydrate fractions (mg/g dry matter) in the wood
of branches inoculated with $\underline{T.\ versicolor}$ in the N x W experiment.†

Treatment	Soluble sugars	Starch		Available carbohydrate	Hemi-cellulose
N_0W_0	14.81	27.03 ⎫		43.84	266.96
N_0W_1	15.32	29.01 ⎭ 28.02		44.33	278.24
N_1W_0	17.87	29.90 ⎫		47.77	270.30
N_1W_1	17.29	35.60 ⎭ 32.75		52.89	270.00
N_2W_0	17.46	24.82 ⎫		42.28	273.66
N_2W_1	16.51	35.77 ⎭ 30.30		52.28	263.68

† Each figure is the mean of nine replications.

Orthogonal comparisons

1. Soluble sugars

    (a) N_0 vs N_1, N_2 ***
    (b) N_1 vs N_2 n.s.
    (c) W_0 vs W_1 n.s.

2. Starch

    (a) N_0 vs N_1, N_2 n.s.
    (b) N_1 vs N_2 n.s.
    (c) W_0 vs W_1 **

3. Total available carbohydrate

    (a) N_0 vs N_1, N_2 *
    (b) N_1 vs N_2 n.s.
    (c) W_0 vs W_1 **

4. Hemicelluloses

    (a) N_0 vs N_1, N_2 n.s.
    (b) N_1 vs N_2 n.s.
    (c) W_0 vs W_1 n.s.

with no significant difference between the latter. Water stress had no significant effect on the levels of soluble sugars.

Nitrogen treatments had no influence on the amount of starch in the 3-year-old wood with mean levels of 28.02, 32.75 and 30.30 mg./g. for treatments N_0, N_1, N_2 respectively (Table 20). However, in water stressed trees there was significantly less starch in the wood, particularly in the vigorously growing N_1 and N_2 trees. The decrease in starch level was not compensated for by a concomitant rise in the quantity of soluble sugars. Statistical analyses of the quantities of available carbohydrates (combined soluble sugars and starch), showed that the main effect of nitrogen deficiency and water stress was to reduce the available carbohydrate in the inoculated wood. The level of hemicelluloses in wood appeared unaffected by either nitrogen treatment or water stress.

Magness et al. (1932) and Mochizuki (1963) reported decreased starch levels in tissues of apple trees subjected to water stress. The former workers also reported increased soluble sugar levels under water stress conditions. There are few specific reports dealing with the effects of nitrogen treatment on carbohydrates in apple. Yokomizo et al. (1964) reported that carbohydrate resources tended to accumulate in 5-year-old Jonathon trees under nitrogen deficiency. In the present study, nitrogen treatment did not alter the starch or hemicellulose content of the 3-year-old wood, but nitrogen deficiency caused a small reduction in the amounts of soluble sugars in the wood compared with normal and high nitrogen treatments. Yokomizo et al. (1964) analysed bark and wood together, which may have obscured differences between levels of carbohydrates in the bark and wood and could account for the variation between their results and those observed in the in the present study.

(ii) Leaf analyses.

The mean levels of five major elements in leaf samples taken from inoculated trees during the season of inoculation are shown in Table 21. The data on which Table 21 is based is given in Appendix 2(e). Increasing nitrogen supply led to significant increases in leaf nitrogen at each of the three nitrogen levels used. Increasing nitrogen level significantly increased leaf calcium and magnesium between N_0 and N_1, N_2 levels but not between the N_1 and N_2 levels. Leaf potassium decreased with increasing nitrogen supply, although only the differences between N_0 and N_1, N_2 were significant. Yokomizo et al. (1964) observed similar results. Nitrogen supply had little effect on leaf phosphorus, a result which contrasts with that in the wood and bark, where phosphorus level increased with nitrogen supply (Tables 18 and 19). The mean leaf levels of phosphorus in trees of all treatments except the N_0W_1 treatment were quite similar (Table 21).

The leaf nitrogen content of water stressed trees was not significantly different from that in trees watered regularly. Leaf phosphorus was significantly less in water stressed trees, a result in contrast with that found in the wood and bark but in agreement with Mason (1958), Hibbard and Nour (1959) and Goode and Hyrycz (1964). Leaf contents of K and Ca were reduced in W_0 trees but Mg content was not affected, compared with the W_1 trees.

With reference to the objectives of the experiment, the nutritional differences measured, thought most likely to be of significance in relation to T. versicolor infections, were those involving the nitrogen, phosphorus and carbohydrate content of the wood. These differences may be summarised as follows: (a) the differences in the nitrogen and

TABLE 21

The mean levels of five major elements in the leaves of trees inoculated with $\underline{T.\ versicolor}$ during the period of inoculation† (samples taken 30-1-70).

Treatment	N	K	Ca	P	Mg
	per cent dry matter			ppm dry matter	
N_0W_0	1.27	1.75	0.29	1620	1740
N_0W_1	1.44	2.46	0.39	2120	1750
N_1W_0	2.21	1.83	0.45	1490	2260
N_1W_1	2.15	2.10	0.58	1670	2470
N_2W_0	2.67	1.78	0.48	1510	2340
N_2W_1	2.64	1.91	0.57	1610	2500

† Each figure is the mean of nine replications.

Orthogonal comparisons

1. Nitrogen
   - (a) N_0 vs N_1, N_2 ***
   - (b) N_1 vs N_2 ***
   - (c) W_0 vs W_1 n.s.

2. Potassium
   - (a) N_0 vs N_1. N_2 ***
   - (b) N_1 vs N_2 n.s.
   - (c) W_0 vs W_1 ***
   - (d) W_0 vs W_1 at N_0 vs N_1, N_2 **
   - (e) W_0 vs W_1 at N_1 vs N_2 n.s.

3. Calcium
   - (a) N_0 vs N_1, N_2 ***
   - (b) N_1 vs N_2 n.s.
   - (c) W_0 vs W_1 ***

4. Phosphorus
   - (a) N_0 vs N_1, N_2 **
   - (b) N_1 vs N_2 n.s.
   - (c) W_0 vs W_1 **

5. Magnesium
   - (a) N_0 vs N_1, N_2 ***
   - (b) N_1 vs N_2 n.s.
   - (c) W_0 vs W_1 n.s.

phosphorus contents of the 3-year-old inoculated wood, between the three nitrogen treatments applied. Mean nitrogen contents of wood were $N_0 = 0.25\%$, $N_1 = 0.34\%$ and $N_2 = 0.40\%$ and mean phosphorus contents were $N_0 = 475$ ppm, $N_1 = 607$ ppm and $N_2 = 676$ ppm; (b) the decrease in starch levels of inoculated wood under water stress. In W_0 trees the mean content was 26.95 mg./g. and in the W_1 trees 33.46 mg./g.

2. <u>Bark symptoms on limbs inoculated with T. versicolor.</u>

Two types of bark symptoms were observed on the inoculated branch stubs.

(a) Papery bark.

The nature and development of papery bark was described in Section I. Most papery bark occurred in the 3-4 months immediately after inoculation. No extension of papery bark symptoms was observed during summer. On a few stubs, limited development of papery bark was observed in the late autumn and winter. Plate 43 shows an example of papery bark.

(b) Physiological dieback.

This symptom received brief mention in Section II. The term physiological dieback was used to describe the symptom because it was found to occur on branch stubs uninoculated with <u>T. versicolor</u>. The symptom developed only during the summer period. The first indication of the development of physiological dieback was a collapse of the bark, usually along a well defined boundary between healthy and affected bark. The initial collapse of the bark occurred at a variable distance between the point of wounding and the first active node. After this the bark normally died and shrank onto the wood (Plate 44). In some cases the bark, although sunken, remained green and partially alive. Physiological dieback differed from papery bark in mode of formation and final appearance.

PLATE 43   Papery bark on the inoculated stub of
a tree in the N_2W_0 treatment, twelve
months after inoculation.

PLATE 44   An inoculated branch stub on a tree of the
N$_2$W$_1$ treatment showing a small area of papery
bark and a more extensive area of physiological
dieback beneath the papery bark, twelve months
after inoculation.

The wood underlying areas of physiological dieback was discolored, with abundant gum in the vessels.

The occurrence and mean length of the bark symptoms are shown in Table 22. A feature of the results was the lack of occurrence of papery bark on the nitrogen deficient trees (Plate 45). Papery bark occurred on all the inoculated stubs in the N_1 and N_2 treatments, either in the presence or absence of water stress, although the mean lengths of the lesions were less than 1 cm. There were no significant differences in the length of papery bark between these four treatments. The results confirmed those of Wade (1968), that papery bark did not occur on nitrogen deficient trees. On the criterion of papery bark symptoms, the trees receiving normal and high nitrogen levels were more susceptible to T. versicolor than the nitrogen deficient trees.

Physiological dieback was very variable in its occurrence, both within and between treatments (Table 22). The irregular occurrence of the symptom prevented valid statistical analysis. Trees of the two nitrogen deficiency treatments showed little physiological dieback. Three of the eighteen stubs showed symptoms in the N_0 treatments, compared with ten and twelve in the N_1 and N_2 treatments respectively. The W_1 trees showed a higher incidence of physiological dieback than W_0 trees (16 and 9 respectively). The length of physiological dieback was more extensive than that of papery bark (Table 22).

Papery bark and physiological dieback occurred on the same stubs but scatter diagrams of length of physiological dieback versus length of papery bark showed no relationship between the two types of symptom. Similarly, there was no apparent relationship between the length of

TABLE 22

The occurrence and mean length of two types of bark symptoms
observed on 3-year-old limbs inoculated with T. versicolor
in the N x W experiment.

Treatment	N_0W_0	N_0W_1	N_1W_0	N_1W_1	N_2W_0	N_2W_1
Papery bark						
No. of stubs showing symptoms	0^+	0	9	9	9	9
Mean length per treatment (cm.)	0.0	0.0	0.8	0.9	0.9	0.9
Physiological dieback						
No. of stubs showing symptoms	1	2	4	6	4	8
Mean length per treatment (cm.)	0.3	0.3	1.9	2.2	1.2	3.3
Mean length per stub showing sympt. (cm.)	2.2	1.2	4.2	3.6	2.8	3.7
Mean length from point of inoculation to first active node 1969-70 season (cm.)	6.8^{++}	6.7	6.9	7.7	7.5	7.5

$^+$ Nine replications per treatment.

$^{++}$ Differences amongst the treatment means were not significant at
0.1% probability.

PLATE 45   An inoculated branch stub on a tree
of the N_0W_0 treatment, twelve months
after inoculation. The bark of the
stub appeared healthy.

papery bark and the distance to the nearest active node during the period of inoculation.

Physiological dieback developed on the stubs during the summer, after the formation of papery bark. This, together with the observation that physiological dieback never extended past the first active node, indicated that the extent of physiological dieback was probably controlled by the distance from the point of wounding or the maximum extent of papery bark to the first active node. A scatter diagram of length of physiological dieback versus length of symptom-free bark (length from point of wounding to the first active node - length of papery bark) for the N_1 and N_2 treatments indicated a linear relationship existed between the length of physiological dieback and length of symptom-free bark (Figure 10). This was most evident in the N_1W_1 and N_2W_2 treatments where there was a high incidence of physiological dieback. Inconsistencies in the relationship between length of symptom-free bark and length of physiological dieback appeared to be due to the effects of the treatments on the occurrence of physiological dieback. The development of physiological dieback was limited in nitrogen deficient trees and there was a lower incidence of physiological dieback on water stressed trees compared with trees watered regularly. Figure 10 also indicated that there was a threshold value for the occurrence of physiological dieback and that it only developed when the length of symptom-free bark was more than 3 cm.

FIGURE 10

Length of physiological dieback versus length of symptom-
free bark (length from point of wounding to first active
node -- length of papery bark) for inoculations on trees of
the N_1W_0, N_1W_1, N_2W_0 and N_2W_1 treatments in the N x W
experiment.

LENGTH OF PHYSIOLOGICAL DIEBACK (CM)
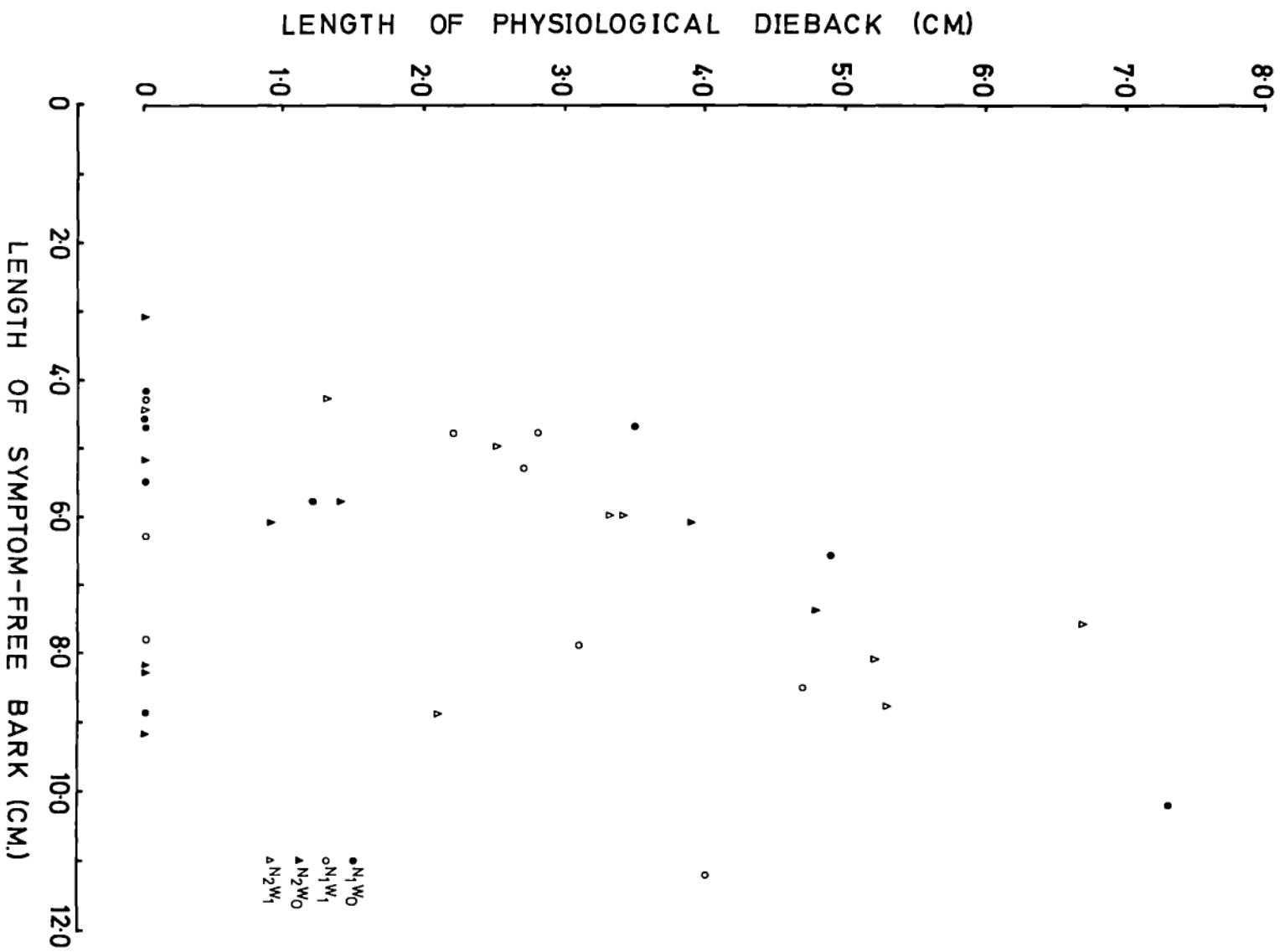
3.   Internal penetration of the stubs by T. versicolor.

The mean length of fungal penetration at the seven points of measurement and the orthogonal comparisons are shown in Table 23. The analysis   of variance of the results is given in Appendix 2(f). Nitrogen treatments had a significant effect on the length of fungal penetration in the stubs. The mean penetration in the N_1 and N_2 treatments was greater (significant at 0.1% probability) than in the N_0 treatments. Lengths of penetration in the N_1 and N_2 treatments were not significantly different. Watering regime had no effect on the penetration of stubs by T. versicolor. Microscopic examination of thin sections revealed hyphal density was similar in all treatments, and isolations showed th⟨ was still viable in all inoculations at the time of harvesting. In this experiment therefore, fungal penetration of the wood was significantly reduced in the nitrogen deficient trees compared with that in the trees of the N_1 and N_2 treatments. This result was in agreement with the observation made previously from measurements of papery bark development, that the trees receiving normal and high nitrogen supply were more susceptible to T. versicolor than the nitrogen deficient trees.

The relation between internal penetration of stubs by T. versicolor and the length of (external) symptoms was also considered. In the N_1 and N_2 treatments, the mean length of papery bark was approximately the same as the mean internal penetration in the youngest xylem (positions 1,1 Table 23). In the N_0 treatments however a mean penetration of 0.4 cm. occurred in the youngest xylem without any development of papery bark. In fact, the bark overlying these areas appeared normal (Plate 45). It seemed therefore, that the formation of

TABLE 23

Mean length (cm.) of internal penetration of branch stubs by T. versicolor
at seven points across their diameter, 12 months after inoculation,
in the N x W experiment.

| Treatment | Position of measurement | | | | | | | Mean length of penetration per stub in each treatment | Mean length papery bark per N treatment |
	1	2	3	4 Pith	3	2	1		
N_0W_0	0.43	0.83	0.80	0.56	0.88	0.84	0.47	0.53	
N_0W_1	0.33	0.83	0.94	0.67	1.02	0.84	0.42	0.56	
Mean penetration N_0 treatment	0.38	0.83	0.87	0.62	0.95	0.84	0.44	0.55	0.0
N_1W_0	0.71	2.10	2.16	1.27	1.46	1.40	0.74	1.09	
N_1W_1	0.64	1.29	1.53	1.32	1.63	1.20	0.71	0.92	
Mean penetration N_1 treatment	0.68	1.70	1.85	1.30	1.55	1.30	0.72	1.00	0.85
N_2W_0	0.83	1.37	1.22	1.12	1.30	1.16	0.79	0.87	
N_2W_1	0.80	1.26	1.36	1.17	1.22	1.11	0.74	0.85	
Mean penetration N_2 treatment	0.82	1.32	1.29	1.15	1.26	1.14	0.76	0.86	0.90

Orthogonal comparisons

    (a) N_0 vs N_1, N_2 ***

    (b) N_1 vs N_2 n.s.

    (b) W_0 vs W_1 n.s.

papery bark was dependent more on the nutritional or physiological condition of the bark than on the precise amount of fungal penetration that had occurred in the wood beneath. Scatter diagrams showed no relationship between the mean internal penetration per stub (mean length of penetration in seven positions) and the distance to the nearest active node during the period of inoculation.

It was possible that the length of internal penetration could have influenced the amount of physiological dieback that occurred on stubs in the N_1 and N_2 treatments. Scatter diagrams of mean internal penetration per stub or mean length of penetration in the outermost xylem versus physiological dieback showed no relationship between internal penetration and physiological dieback. Thus, in this experiment, the observations and results indicated that physiological dieback differed in development and appearance from papery bark and occurred independently of fungal activity in the wood.

4. Effect of treatments on host reaction.

The gum forming ability of the wood per se was examined using sterile limb sections in vitro as described in Section II. Two-year-old wood from uninoculated trees was used for the test. In incubated limb sections there was no detectable difference in the amounts of gum formed in the wood from trees of any of the six treatments.

In vivo, discoloration and gum formation were restricted to a 0.5-1.5 cm. zone in advance of fungal penetration, in the stubs on which no physiological dieback occurred. Measurements on two replications of each treatment, showed the extent of the gum zone was similar in all treatments (1.0-1.4 cm.). Though assessed visually, the

intensity of gum formation and the morphology of the gum in the vessels appeared similar in all treatments.

Physiological dieback was symptomatic of changes in the wood underlying the affected bark. In inoculations which showed physiological dieback, the wood beneath the bark was discolored and the vessels filled with gum. The gum zone was extended to the limit of physiological dieback.

(B) Effect of phosphorus nutrition on the susceptibility of young STP trees to T. versicolor.

1. Effect of treatments on tree growth and nutritional status.

(a) Leaf symptoms and growth effects.

The trees received the phosphorus treatments for three seasons -- two growing seasons prior to inoculation and for the season during the period of incubation with T. versicolor. The first leaf symptoms of phosphorus deficiency became visible during the second growing season and continued to develop in severity over the third season. Leaf symptoms however, were never very prominent. Mostly leaves showed a bronzing with some interveinal purpling. The leaves of P_0 trees were smaller and more leathery in texture than those of P_1 or P_2 trees. P_0 trees defoliated earlier than trees in the P_1 and P_2 treatments.

The effects of phosphorus deficiency on growth were more obvious. Shoot growths were thinner and fewer than on P_1 or P_2 trees and secondary growth was much reduced (Plate 46 a and b). In addition, earlier cessation of the growth of P_0 trees was observed. The effects of phosphorus supply on length of shoot growth per tree and weight of prunings per tree over three seasons are shown in Table 24. The full data is given in Appendix 2(g).

PLATE 46   (a)   Left - tree of P_0 treatment
(b)   Right - tree of P_2 treatment.
Note general reduction in shoot and secondary
growth of the tree in the P_0 treatment.
(Photographed during the second season of
treatment application, February 1970.)

TABLE 24

The mean lengths of shoot growth and weights of prunings
per inoculated tree for three seasons in the P experiment.[†]

Season		P_0	P_1	P_2
1968-69	Mean shoot length per tree (cm.)	573	760	783
	Mean wt. prun. per tree (g.)	195	310	334
1969-70	Mean shoot length per tree (cm.)	820	1554	1760
	Mean wt. prun. per tree (g.)	92	226	245
1970-71	Mean shoot length per tree (cm.)	623	1492	1522
	Mean wt. prun. per tree (g.)	58	174	181

[†] Each figure is the mean of 12 replications.

Orthogonal comparisons

1. Shoot lengths

    (a) 1968-69 P_0 vs P_1, P_2 **
                          P_1 vs P_2 n.s.

    (b) 1969-70 P_0 vs P_1, P_2 ***
                          P_1 vs P_2 *

    (c) 1970-71 P_0 vs P_1, P_2 ***
                          P_1 vs P_2 n.s.

2. Weight of prunings

    (a) 1968-69 P_0 vs P_1, P_2 ***
                          P_1 vs P_2 n.s.

    (b) 1969-70 P_0 vs P_1, P_2 ***
                          P_1 vs P_2 n.s.

    (c) 1970-71 P_0 vs P_1, P_2 ***
                          P_1 vs P_2 n.s.

TABLE 25

The mean leaf contents of five major elements in trees
supplied with three levels of phosphorus in the 1969-70 season.
(Samples taken 30-1-70). Mean leaf phosphorus levels for the
1970-71 season are also shown.†

Treatment	N	K	Ca	Mg	P (1969-70)	P (1970-71)
	Per cent dry matter			Ppm dry matter		
P_0	1.82	2.13	0.60	2220	790	.720
P_1	1.91	1.95	0.85	2710	1350	2050
P_2	2.06	1.97	0.81	2620	1850	3180

† Each figure is the mean of 12 replications.

Full data is given in Appendix 2(h).

Orthogonal comparisons

			1969-70	1970-71
1.	Phosphorus	P_0 vs P_1, P_2	***	***
		P_1 vs P_2	***	***
2.	Nitrogen	P_0 vs P_1, P_2	***	
		P_1 vs P_2	*	
3.	Potassium	P_0 vs P_1, P_2	n.s.	
		P_1 vs P_2	n.s.	
4.	Calcium	P_0 vs P_1, P_2	***	
		P_1 vs P_2	n.s.	
5.	Magnesium	P_0 vs P_1, P_2	**	
		P_1 vs P_2	n.s.	

The results showed that there was a progressive reduction in growth each season under phosphorus deficiency conditions. The reduction in growth was more strongly reflected in the weight of prunings than in the length of shoot growth, indicating the important effect of phosphorus on secondary growth. Growth was not significantly increased at the high phosphorus level (P_2) compared with the normal level (P_1).

(b) Nutritional status of the trees at inoculation and during the period of ~~inoculation~~ incub-ation.

(i) Leaf analyses.

Analysis of leaf samples over three seasons showed there was a continual decline in leaf phosphorus status under conditions of phosphorus deficiency. During 1968-69, analyses showed mean leaf phosphorus levels of 1200 ppm (P_0), 1700 ppm (P_1) and 2100 ppm (P_2). In P_0 trees in 1969-70 the mean level had dropped to 790 ppm (Table 25) and to 720 ppm in 1970-71. Why the leaf phosphorus level only declined marginally in 1970-71 is not clear, but it is possible that the leaf phosphorus level was maintained at the expense of phosphorus reserves in the wood and bark. High phosphorus supply (P_2) resulted in increased phosphorus concentrations in the leaves, in each season, compared to P_1 treatments (Table 25). Phosphorus concentration in leaves rose considerably in both P_1 and P_2 treatments in 1970-71 compared with the previous season, particularly in the P_2 treatment. The difference in phosphorus status for the trees of the same treatment in different seasons, suggested a strong seasonal effect on leaf phosphorus status. Phosphorus supply also influenced the leaf concentration of other elements. Increasing phosphorus supply increased nitrogen, calcium and magnesium levels but decreased potassium concentration.

(ii) Nutritional status of inoculated limbs.

Nitrogen and phosphorus.

The mean values for nitrogen and phosphorus analyses of samples of bark and wood from inoculated limbs (2-years-old) are shown in Table 26 and the full results in Appendix 2(i). Both the wood and bark phosphorus contents were significantly different between the three phosphorus treatments, at the time of inoculation. On a proportional basis the relative phosphorus contents of the wood and bark remained similar under phosphorus deficiency conditions. The mean phosphorus content of the wood of P_0 trees declined to 58% of the control level while the mean phosphorus content of the bark of P_0 trees declined to 51% of the control level. Phosphorus deficiency had resulted in a significant drop in bark nitrogen level but wood nitrogen level had not been affected. Analyses of 2- and 3-year-old wood during the period of inoculation [Appendix 2(j)] showed a seasonal cycle of depletion and replenishment of wood phosphorus reserves. Although it was not possible to inoculate 3-year-old wood due to the growth pattern of the trees, 3-year-old wood from uninoculated trees was found to be only marginally lower in phosphorus status than the 2-year-old wood.

Thus the treatments (P_0, P_1, P_2) were effective in establishing a differential in the phosphorus content of the wood and the bark of the experimental trees.

Carbohydrate status.

Inoculated wood in P_0 trees was significantly lower in soluble sugars and starch than the wood of P_1 or P_2 trees (Table 26). The decrease in starch level in P_0 trees was greater than the fall in soluble sugars.

TABLE 26

The mean levels of nitrogen, phosphorus and carbohydrates in the bark
and wood of 2-year-old limbs inoculated with T. versicolor in the
P experiment.†

Tissue	Factor	P_0	P_1	P_2
WOOD	N content (% d.m.)	0.19	0.19	0.20
	P content (ppm d.m.)	227	392	450
	Soluble sugars (mg./g. d.m.)	15.78	17.59	17.30
	Starch (mg./g. d.m.)	30.66	36.12	36.63
	Tot. avail. carbohydrate	46.44	53.71	53.93
	Hemicelluloses (mg./g. d.m.)	248.00	253.20	254.60
BARK	N content (% d.m.)	0.91	1.07	1.07
	P content (ppm d.m.)	927	1803	2125
	Soluble sugars (mg./g. d.m.)	55.65	68.03	69.10
	Starch (mg./g. d.m.)	41.00	42.00	42.95
	Tot. avail. carbohydrate	96.65	110.03	112.05

† Each figure is the mean of 12 replications.

Orthogonal comparisons	wood	bark
1. Phosphorus content		
P_0 vs P_1, P_2	***	***
P_1 vs P_2	*	**
2. Nitrogen content		
P_0 vs P_1, P_2	n.s.	***
P_1 vs P_2	n.s.	n.s.
3. Soluble sugars		
P_0 vs P_1, P_2	*	***
P_1 vs P_2	n.s.	n.s.
4. Starch		
P_0 vs P_1, P_2	***	n.s.
P_1 vs P_2	n.s.	n.s.
5. Available carbohydrate		
P_0 vs P_1, P_2	***	***
P_1 vs P_2	n.s.	n.s.
6. Hemicelluloses		
P_0 vs P_1, P_2	n.s.	-
P_1 vs P_2	n.s.	-

Hemicellulose levels in the wood appeared unaffected by phosphorus treatment. Data on which Table 26 is based is given in Appendix 2(k).

In the bark, the decrease in soluble sugar level was particularly marked in the P_0 treatment (Table 26). Starch levels in all treatments were very similar. The overall result of these changes was to reduce the levels of available carbohydrate (combined soluble sugars and starch) in both the wood and bark.

The carbohydrate levels of 2- and 3-year-old wood from the uninoculated trees of the different phosphorus treatments were similar. The same relative seasonal changes in carbohydrate reserves were observed in trees of the three phosphorus treatments.

Thus, there had been a decline in the available carbohydrate content of wood and bark under phosphorus deficiency conditions. Little information exists on the effects of phosphorus deficiency on carbohydrate resources in perennial plants and the author knows of no specific reference to apples. In other plants variable effects of phosphorus deficiency on carbohydrate resources have been reported. Much of the relevant work has been reviewed by Singh and Singh (1968) and Reid and Bieleski (1970). The effects observed differed with plant species, plant part and the carbohydrate fraction involved.

2.   Bark symptoms on limbs inoculated with T. versicolor.

As in the experiment dealing with nitrogen level and watering regime, two types of bark symptoms were observed - papery bark and physiological dieback.

Limbs were inoculated in July 1970 and the first papery bark symptoms were observed approximately six weeks after inoculation.

Papery bark developed in all treatments, although the occurrence of papery bark was much greater both in extent and number of inoculations on which it occurred, in the P_0 treatment (Table 27). The development of papery bark had ceased in all treatments by December 20, 1970. The length of papery bark development at this time was compared with the distance to the active node from the point of inoculation (Figure 11). In the P_0 treatments, there was a close relationship between the two, the length of papery bark tending to increase until it reached the first active node. In the P_1 and P_2 treatments, where the occurrence of papery bark was much more variable, this relationship did not hold. Thus, the length from the point of inoculation to the first active node appeared to control the extent of the initial papery bark development that occurred on the inoculated limbs of trees in the P_0 treatment. However, phosphorus deficiency predisposed bark to a more extensive development of papery bark than occurred on trees in the normal and high phosphorus treatments.

After December 1970 and during the summer and autumn period until April 1971, a small amount of physiological dieback occurred (Table 27). It was restricted to inoculations on trees in the P_1 and P_2 treatments. The amount of physiological dieback that occurred in this experiment was much smaller than the amount in the N x W experiment where the distance from the point of inoculation to the first active node was greater. As in the nitrogen and water experiment physiological dieback developed on inoculations which showed papery bark as well as those that did not. No physiological dieback occurred on the P_0 trees but this was probably because in most cases, papery bark already extended to the first active node.

TABLE 27

The occurrence and mean length of two types of bark symptoms
at three times after inoculation on 2-year-old inoculated limbs
in the P experiment.

Time and parameter	Treatment			Orthogonal comparison
	P_0	P_1	P_2	
1. 20-12-70				
No. inoc. showing papery bark (max. no. 24)	24	14	10	
Mean length papery bark per inoculation	2.0 cm	0.9 cm	0.4 cm	P_0 vs P_1, P_2***
2. 20-4-71				
No. inoc. showing physiological dieback	0	11	16	
Mean length physiological dieback per inoculation	0.0 cm	0.4 cm	0.8 cm	
3. 20-6-71				
No. inoc. showing new development of papery bark	0	4	6	
Total no. inoc. which showed some papery bark at harvest	24	18	16	
Mean length papery bark per inoc. at harvest	2.0 cm	1.1 cm	0.8 cm	
Mean length total bark symptoms (physiol. dieback + papery bark) per treatment)	2.0 cm	1.5 cm	1.6 cm	
Mean length from wound to nearest active node	2.0 cm	1.7 cm	1.7 cm	Diff. between treatments n.s. at 0.1% probability.

FIGURE 11

The length of papery bark at December 20, 1970 versus
the distance from the point of inoculation to the first
active node, for inoculations in the P_0, P_1 and P_2
treatments.
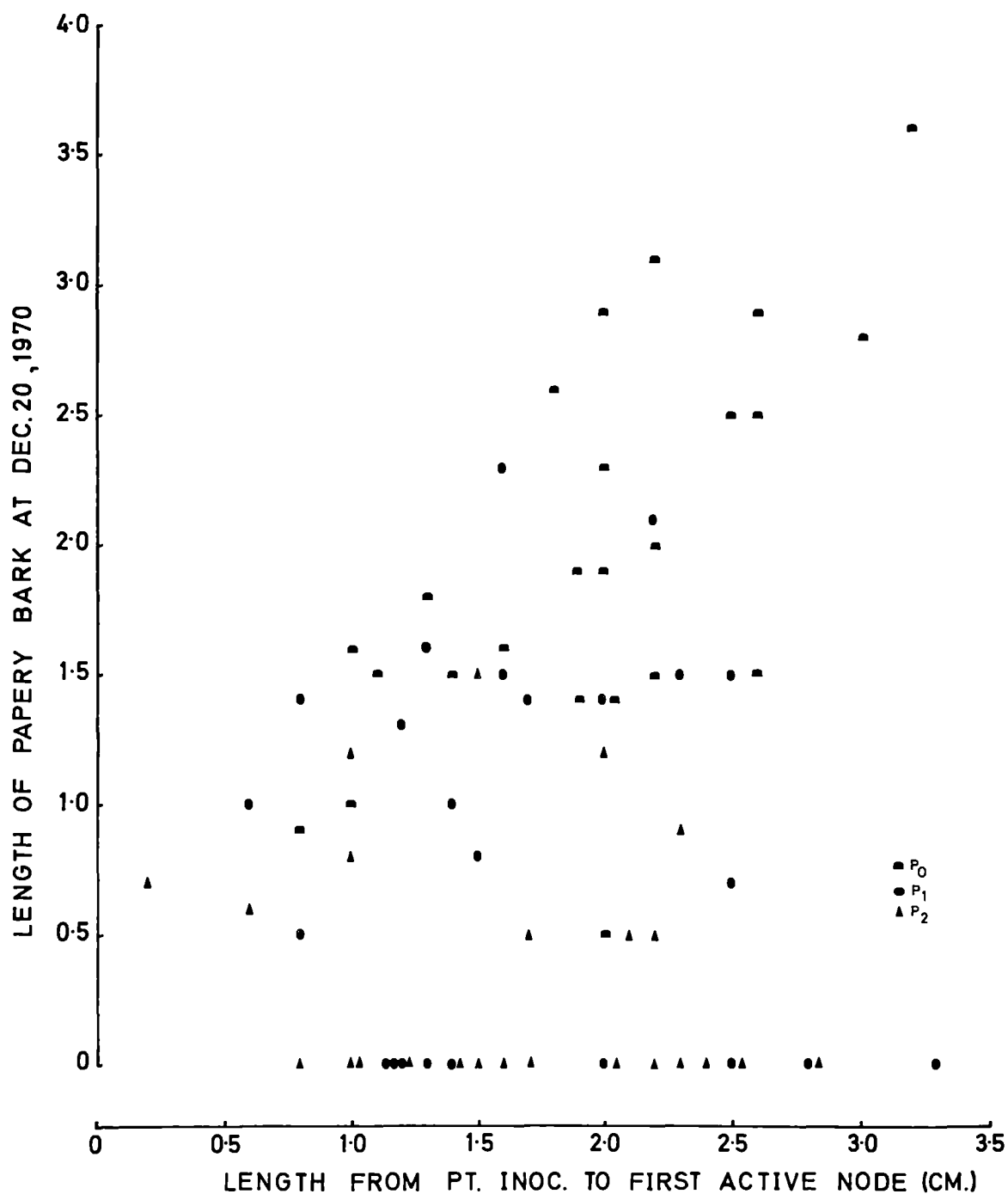
No further extension of the bark symptoms was observed after April 1970, until immediately prior to the final harvesting in June 1970, when further development of papery bark took place on some inoculations in the P_1 and P_2 treatments (Table 27). The new papery bark extended from the margin of previous symptoms (papery bark or physiological dieback) and developed in the normal manner with swelling, splitting and callus outgrowths (Plate 47). The papery bark formation extended for 1-3 cm. In all cases except one it extended only as far as the first active node of the previous growing season. The development of papery bark had ceased by the time of harvesting. The re-occurrence of active papery bark formation on established inoculations during the winter, gives strong support to the observation made in Section I that papery bark had a specific seasonal occurrence.

As in the N x W experiment there was no apparent relationship between the two types of bark symptoms observed. With the additional bark death that occurred after the initial papery bark development, most inoculated limbs showed either or both papery bark or physiological dieback (Table 27). A scatter diagram of the length of physiological dieback versus length of symptom-free bark (length to first active node - length of papery bark development until December 1970) showed a linear relationship between the two (Figure 12). The relationship was more consistent than observed in the N x W experiment and the development of physiological dieback did not appear to be dependent on a minimum length of symptom-free bark (compare Figures 10 and 12).

Another feature noted on a number of inoculated stubs (3 in P_1 treatment, 5 in P_2 treatment) was a black sooty mold type growth (Plate 48). The growth was restricted to stubs which had been sheltered by other

PLATE 47   Left: papery bark on an inoculated stub of a
tree in the P_0 treatment. The papery bark
developed between the time of inoculation
(July 1970) and December 1970.

Centre: examples of the papery bark which
developed on a number of inoculated stubs
of trees in the P_1 and P_2 treatments,
immediately prior to harvesting in June 1971.

Right: longitudinal section of an inoculated
stub from a tree of the P_0 treatment showing
the white rot which had occurred, and the
normally restricted discoloration in advance
of fungal decay.

FIGURE 12

Length of physiological dieback versus the length of
symptom-free bark (length from point of wounding to the
first active node - length of papery bark) at April 20,
1970 for inoculations on trees of the P_1 and P_2 treatments.
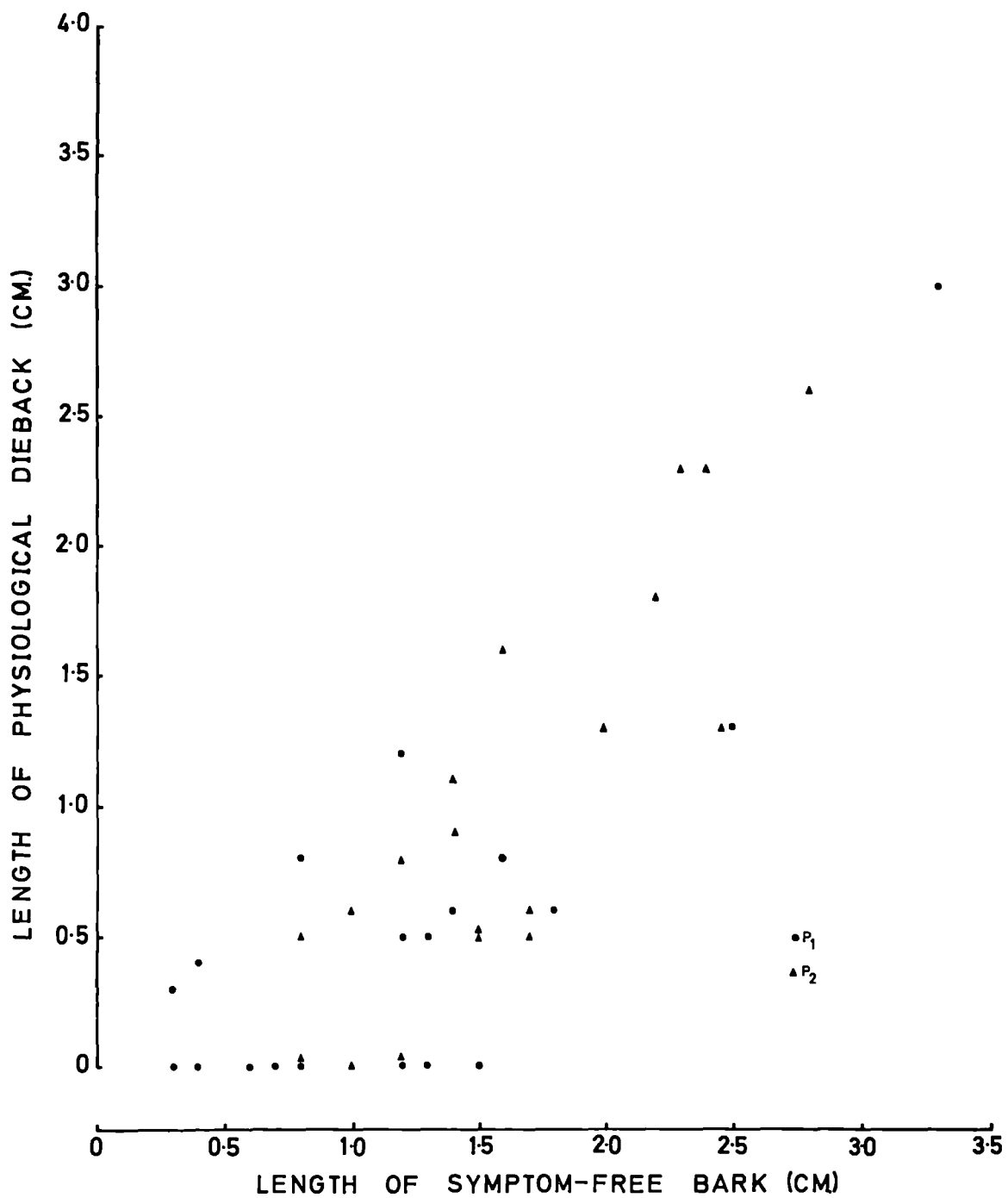
PLATE 48   Right: illustrating the black fungal growth
            which occurred on the bark and wound
            surface of some inoculated stubs in the P_1
            and P_2 treatments. (Photographed eleven
            months after inoculation.)

            Left: longitudinal sections of stubs which
            showed external fungal growth. Note the
            extensive discoloration of the sapwood in
            advance of fungal decay.

branches and leaves during the period of inoculation and the stubs other-wise showed normal bark symptoms. The growth was mainly fungal with Alternaria and Coniothecium sp. being two of its main components. In Western Australia, Doepel (1962) used the term 'ink stain' to describe a similar phenomenon on apple limbs attacked by wood rotting fungi.

3. Internal penetration of stubs by T. versicolor.

The mean lengths of internal penetration in inoculated limbs are shown in Table 28. There were no significant differences between treatments for internal penetration of stubs. This result contrasted with that obtained from measurements of length of papery bark development which indicated phosphorus deficient trees were more susceptible to T. versicolor than trees of the P_0 and P_1 treatments.

The average penetration in the outermost xylem (positions 1, 1) was greater in the P_0 treatments than P_1 or P_2 treatments, but penetration in the inner xylem and pith was greater in the P_1 and P_2 treatments than in the P_0 treatments. In many inoculations, maximum hyphal pene-tration occurred more frequently in the pith than observed in other experiments. Isolations showed the fungus to be viable in all inoculations at harvest time.

The mean length of fungal penetration in the outermost xylem was greater than the combined mean length of physiological dieback and papery bark (Tables 27 and 28). This indicated that the fungus may have influenced the occurrence of physiological dieback. However, it was not possible to deduce any relationship between fungal penetration and physiological dieback, and it seemed that the physiological dieback probably occurred before the penetration of the fungus. The discrepancy

TABLE 28

Mean length (cm.) of internal penetration by $\underline{T}$. $\underline{versicolor}$
of branch stubs at seven points across their diameter,
10 months after inoculation in the P experiment.

Treatment	Position of measurement						
	1	2	3	4 Pith	3	2	1
P_0	2.4	3.0	3.1	3.3	3.2	3.1	2.6
P_1	1.9	3.6	3.5	3.5	3.5	3.4	2.0
P_2	1.9	4.0	4.0	4.0	4.0	3.8	2.2

Orthogonal comparisons

(a) P_0 vs P_1, P_2 n.s.

(b) P_1 vs P_2 n.s.

between the maximum length of bark symptoms and the internal penetration in the outer xylem, indicated some bark remained alive and symptomless even though the wood beneath was physiologically altered, either by discoloration or decay. A similar situation was observed in the N x W experiment. The papery bark which developed prior to harvest appeared to form in bark overlying discolored wood. The maximum internal hyphal penetration in limbs, was greater in practically all instances, than the distance from the point of inoculation to the first active node. Sufficient outer xylem tissue remained healthy for the shoot from the node to maintain active growth.

Inoculations noted to have external fungal growth on the bark of the stub and the inoculated surface, showed greater internal fungal penetration than the mean values for the P_1 or P_2 treatments. This difference was sometimes as great as 30-40 mm. The greater internal penetration cound have been related to the extensive discoloration and lack of gum formation in the wood in advance of the fungus in these inoculations (see below).

4. <u>Effect of treatments on host reaction.</u>

The extent of gum formation was examined in ten inoculations selected at random from the P_0 and P_1 treatments. Visually there appeared no difference between treatments with regard to the morphology of gum deposits and intensity of gum formation, and measurements failed to show any differences in extent of the gum zone (mean length 1.3 cm. in the P_0 treatment and 1.5 cm. in the P_1 treatment). Incubation of limb sections from uninoculated trees <u>in vitro</u> failed to show any differences in gum forming ability of wood from any treatment.

Normally, discoloration in advance of T. versicolor infections in young wood was restricted to 0.5-1.5 cm. (Plate 47). However, in stubs in the P_1 and P_2 treatments which showed external fungal growth, discoloration in the wood extended well in advance of the maximum point of fungal penetration (Plate 48). The discolored wood was light-brown and took the form of a central column with distinct margins between healthy and discolored wood. The discoloration usually extended through the 2-year-old wood (inoculated) into the 3-year-old wood, a distance of from 4-12 cm.

The ray and xylem parenchyma cells of this abnormally extensive zone of discolored wood were devoid of starch but little gum was present in the vessels. A narrow zone of gum marked the border between healthy and affected wood. The cells at the maximum extent of discoloration normally appeared devoid of contents. At the maximum extent of fungal penetration, and usually for a good distance in advance, the cells in the discolored wood contained yellow-brown globules. These globules were found to stain red with the hoepfner-Vorsatz reaction (Plate 49) which indicated they were probably polyphenolic in composition

Investigation revealed that the ray and xylem parenchyma cells lost their starch and showed polyphenol staining increased as the discolored wood was approached from the healthy sapwood. Polyphenol staining reached a maximum immediately prior to the transformation of the cell contents into the globular material. This transformation of cells resembled that previously described in sapwood-discolored wood transition zone (page 61) except for the consistently globular form of the discolored cell contents and the lack of gum in the vessels of wood which normally formed abundant gum.

PLATE 49   Longitudinal section through the sapwood-
           discolored wood transition zone, stained
           for polyphenols. Note the distinctly
           globular nature of the contents of the ray
           and xylem parenchyma in the discolored
           zone, the red staining of the globules
           (positive for polyphenols) and the general
           red staining of the ray cells prior to the
           transformation of their contents into
           globules. x 200.

Examination of a number of uninoculated wounds which had the same type of external fungal growth as shown in Plate 48, revealed a similar discoloration extending from the wound surface deep into the wood. It appeared therefore, that the fungi growing on the wound surface produced some xylem translocatable material which caused the death and discoloration of the host tissue but which did not affect (possibly even stimulated) penetration by T. versicolor. This latter contention was supported by the fact that there was no antagonism in mixed culture between T. versicolor and several of the fungi isolated from the bark surface.

DISCUSSION

Discussion

Trametes (Polystictus) versicolor is normally a saprophyte on dead wood and confusion has existed as to its role as a parasite. Observations made in this study indicate T. versicolor should be considered as a facultative parasite. Blumer and Neusch (1962) reported that T. versicolor could grow as a commensal or saprophyte in the pith of cherry and apple limbs. Wade (1968) claimed that apple trees varied in their susceptibility to T. versicolor and that the fungus could act as a parasite or as an endophyte. In Western Australia, Doepel (1965) observed that the wood rot of apple trees caused by T. versicolor and other species could be a secondary phenomenon, and that the fungi attacked parts of the trees which were already dead. Evidence obtained in this study indicates that in Tasmania, T. versicolor is a primary parasite which attacks the living sapwood of trees, and that the fungus is capable of progressive infection and decay of the sapwood. Penetration of sapwood by T. versicolor is accompanied by a white rot of the wood although bark symptoms may not be present, and the fungus does not grow as an endophyte. T. versicolor was not observed to decay the heartwood or dead sapwood portions of living trees. However, heartwood was of rare occurrence in field trees and the incidence of attack of dead sapwood portions was probably limited because the decline and death of limbs in orchard trees due to physiological causes, is not a serious problem in Tasmania.

Two types of bark symptom occurred on apple limbs infected with T. versicolor. These were termed papery bark and physiological dieback. The latter symptom was only observed on young limbs artificially inoculated with T. versicolor, and its significance will be considered later.

Papery bark was a characteristic symptom on the bark of limbs infected with T. versicolor and initial symptoms involved bark splitting, callus outgrowth and peeling of the phellem. However, evidence indicates that papery bark was a non-specific symptom. Limbs were examined which showed extensive and active papery bark development, but the sapwood of the limbs did not show symptoms of T. versicolor attack and the fungus could not be isolated from the wood. In addition, Howard and Banwell (1969) observed that a papery bark condition developed on young apple trees stored in cool stores, in the presence of apple fruit. Volatiles from the fruit apparently induced the disorder and they suggested ethylene, a major apple volatile of known biological activity, may have been a factor. A range of non-ethylenic volatiles of little known biological functions are also produced by apple fruit (Fidler, 1961).

Papery bark was seasonal in occurrence and it only developed during the autumn and winter. This observation was verified from an examination of papery bark formation on natural and artificial infections. It was not possible to determine whether the effect of season on papery bark development was through an effect on the host or fungal physiological status, or whether external environmental factors such as low temperature and high humidity, controlled the development of papery bark. Evidence that low temperatures may influence the development of papery bark, was the continued development of symptoms on infected limbs stored in a cool room during the spring and early summer. The definite seasonal formation of papery bark contrasts with the observation that the fungus was active in the wood of infected limbs in all seasons. This was evidenced by the studies of fungal penetration in artificial

inoculations, and that it was possible to isolate the fungus from infected limbs at all times of the year.

Nutritional conditions affected the susceptibility of bark to the disorder. The results of Wade (1968) were confirmed, as it was shown nitrogen deficiency prevented the occurrence of papery bark, while phosphorus deficiency made bark more susceptible to the disorder. Papery bark also occurred to a limited extent on healthy trees. The effect of phosphorus deficiency in increasing susceptibility to papery bark is probably an indirect effect. The bark of nitrogen deficient trees was lower in phosphorus than the bark of control trees, but nitrogen deficient trees showed no development of papery bark. On the evidence of the N x W experiment it seems unlikely that the carbohydrate status of the bark is the major factor predisposing bark to papery bark. Soluble sugars declined in the bark of phosphorus deficient trees. While carbohydrate levels in the bark were not determined in the N x W experiment, water stress significantly reduced starch levels in the wood, but trees of the W_0 and W_1 treatments did not differ in their susceptibility to papery bark.

It is possible development of papery bark could be stimulated by physiological changes in the underlying wood, by the fungus itself or by the interaction between host and fungus. Normally, papery bark was associated with discoloration of the underlying wood, but this was not invariably the case as localised areas of bark overlying discolored or decayed wood sometimes remained healthy for long periods or conversely, papery bark extended for some distance over healthy wood. The fungus could produce some biologically active material that stimulates the

development of papery bark. The evidence presented by Howard and Banwell (1969) and cited above, indicates that biologically active materials may be involved in the development of papery bark. That papery bark could be induced by causes other than T. versicolor, and that its development is influenced by specific seasonal and nutritional conditions, indicates that the development of papery bark is mainly dependent on the physiological condition of the tree. It is feasible however, that the activity of the fungus directly or indirectly provides a stimulus for the development of papery bark.

The following hypothesis is proposed to explain the occurrence of papery bark. 'Either the fungus or the disturbed physiology of apple sapwood in advance of T. versicolor infection, results in the production of a biologically active factor(s) that stimulates the development of papery bark. The fungus is either limited in its production of such a material, or the trees seasonal physiological cycle only allows this factor to be produced or expressed by the tree during dormant or semi-dormant periods.' Further research is required to examine active and inactive papery bark zones for types and balance of growth stimulating hormones, the biological activity of material from such zones and the possible biological activity of materials produced by T. versicolor.

The second type of bark symptom observed in this study was referred to as physiological dieback. The symptom and its occurrence were apparently unrelated to papery bark and it occurred in the absence of T. versicolor. This was shown in the N x W experiments and by its occurrence on uninoculated controls. Physiological dieback differed in morphogenesis and appearance from papery bark, and was similar to the

diebacks associated with various mineral deficiency conditions, unfavourable soil moisture status and overcropping in apple. The symptom only occurred during the growing season, and its occurrence seemed an example of the general horticultural observation that pruned shoots tend to die back to the nearest active node. Comparison of the occurrence of physiological dieback in the N x W and P experiments, showed it tended to be greatest in extent when the distance to the active node was longest and the trees were most vigorous. Thus, in the N x W trial, the N_1 and N_2 trees and particularly trees in the N_2W_1 treatment, showed the most physiological dieback. The explanation for the occurrence of physiological dieback, probably lies in the starvation effects produced in the stubs distal to the active node by the basipetal movement of auxins and a lack of nutrients and assimilates (van der Weij, 1932; Jacobs, 1950). Physiological dieback was a whole limb symptom and there was always a close relationship between the extent of bark death and discoloration of the wood underlying the affected bark. There was no evidence that physiological dieback influenced the penetration of branch stubs by T. versicolor.

The pattern of occurrence of physiological dieback and papery bark on artificial inoculations in young wood, places doubts on the reliability of the results of Darbyshire (1967), on the seasonal and varietal susceptibility of apple trees to T. versicolor. That author made inoculations at different seasons and inoculations were left for up to 12 months prior to final symptom measurement. Thus, all inoculations passed through a summer season and could have developed physiological dieback. Darbyshire relied on measurements of bark

symptoms to detect differences in susceptibility and did not differentiate papery bark from physiological dieback. The dieback measured by Darbyshire appeared to be identical to that termed physiological dieback in the present study. In addition, from the author's own short term experiment and observations on the seasonal occurrence of papery bark, it appeared impossible to get a measure of seasonal susceptibility from measurement of bark symptoms on normal field trees.

Measurement of the extent of papery bark was not a reliable means of assessing the susceptibility of trees to T. versicolor. Papery bark developed in autumn and winter while fungal decay of the wood occurred throughout the year. Thus, summer inoculation of young limbs did not produce papery bark symptoms but fungal penetration and decay still occurred. The N x W experiment also illustrated that frequently there was no close spatial relationship between papery bark and internal changes in the affected wood. The most substantial evidence against using papery bark as a sole indicator of susceptibility concerned the results obtained in the P experiment. Trees which received no phosphorus were, on the criterion of papery bark development, the most susceptible to T. versicolor. There was however no significant difference between the extent of fungal penetration in limbs of the trees which received no phosphorus and those supplied with normal-high levels of phosphorus.

_T_. _versicolor_ caused a white rot of apple sapwood. The decay of sapwood by _T_. _versicolor_ was preceeded by the discoloration of the sapwood. That a zone of discolored wood was always present between healthy infected sapwood, indicated discolored wood was a dynamic tissue produced in advance of the fungus, as long as the fungus remained active.

Discoloration of the living sapwood was a non-specific response of the wood to mechanical or pathological stimuli. Results from _in vitro_ experiments with sterile limb sections, showed that only living wood was capable of response to external stimuli, and that the same cellular reactions (loss of starch and nuclei, gum formation and death of ray and xylem parenchyma) occurred in the wood beneath sterile wounds as around natural or artificial infections of _T_. _versicolor_. As well as confirming the non-specificity of the sapwood response, these results also confirmed that discoloration was a definite host reaction (response), and that the products formed in cells of discolored sapwood were host and not fungal in origin.

At the cellular level, there was a consistent spatial relationship between discolored tissue and fungal hyphae. Fungal hyphae were always confined within the zone of discolored wood. In the xlyem, the ray and xylem parenchyma died in advance of the fungal hyphae. The pith was the only tissue where the cells did not die in advance of the fungus. In the pith death of cells seemed dependent on hyphal penetration of the cells. During the decay of the discolored wood both the gum in the vessels and the contents of the dead ray and xylem parenchyma cells were degraded by the fungus. Observations indicate most of the cell contents were broken down prior to any serious attack on the cell walls.

The physiological aspects of discoloration of the sapwood will be considered first. A regular sequence of cytochemical changes occurred in the transformation of sapwood to the discolored state. The end result of such changes was the transformation of the living xylem and ray parenchyma cells from a healthy nucleated condition to one in which the cell lumens were filled with irregular wall encrusting or globular deposits of a yellow-brown material. This latter condition has been described as necrosis by Shain (1967, 1971). The gradual disappearance of starch grains from the cells was one of the most noticeable changes that occurred in the living cells prior to their necrosis. From the limited analyses undertaken, the hydrolysis of starch in the sapwood-discolored wood transition zones was accompanied by a concomitant rise in the soluble sugar level, indicating that sugars formed by the hydrolysis of starch were not translocated away from such areas, a result in agreement with findings in other species (Hillis, 1968). Accompanying the hydrolysis of starch were other changes indicative of a decline in cellular vitality, most noticeably decreased ability of cells to reduce the vital stain TTC and changes in the appearance of nuclei. The cells eventually reached the state where they were completely devoid of starch, TTC reducing ability and nuclei, and appeared only to contain debris, lipid globules and an abnormally high concentration of polyphenols. The ray and xylem parenchyma cells could apparently remain in this condition for long periods (e.g. deep within gum zones) without further change, but usually the cells underwent further transformation to the necrotic condition. It appeared that polyphenols only formed after starch had disappeared from the cells and that, although the cells at the sapwood-discolored

wood boundary did not contain nuclei, enzymes or enzyme systems functioned within the cells to bring about necrosis. The general sequence of events was similar to that observed in heartwood formation in a number of tree species (Frey-Wyssling and Bosshard, 1959).

The extraneous material formed in the necrotic cells was probably oxidised polyphenols (Gagnon, 1967). The strong reaction of cells for polyphenols prior to their necrosis and the partial polyphenol staining of the brown material formed in the cells, supports this suggestion. In addition, the extraction and assay of phenolics showed that the quantity of extractable phenolics, and especially the quantity of low molecular weight phenolics, declined in discolored tissue. This, together with the fact that even prolonged extraction with neutral and alkaline solvents only removed a small proportion of the color of the wood, seems strong evidence that cellular phenolics were oxidised and polymerised to a largely unextractable form in the discoloration process. The coloration of cell walls in discolored wood was presumably due to their impregnation with oxidised phenolics. The substrates for the formation of the extraneous material were probably the cell sugars, lipids and polyphenols. This idea is in agreement with Hillis (1962, 1968) that polyphenolic compounds in heartwood and discolored sapwood are formed _in situ_ from stored or translocated carbohydrate.

A second type of transformation of the pith, ray and xylem parenchyma cells was observed in the sapwood beneath wounds, and on rare occasions in these cells around fungal infections. The cells appeared to die and the starch granules in the cells became aggregated and disorganised _in situ_. On the basis of color and morphology, the material

formed in the cells appeared almost identical to that formed in cells which had first lost their starch by hydrolysis. Neither material stained with specific carbohydrate reagents but the material formed by the aggregation of starch granules did not stain for polyphenols. The material formed by starch aggregation was probably a degraded polysaccharide of the amylodextrin type. Cells in which starch aggregation occurred were normally situated immediately beneath wound surfaces. This indicates the process probably occurred under conditions of slow desiccation, which allowed limited amylase activity but insufficient to bring about complete starch hydrolysis. The similarity in appearance and the inert nature of the two materials, which apparently arise by different physiological processes, suggests that the material formed in cell lumens in cells devoid of starch and nuclei could have a degraded polysaccharide component in addition to its probable phenolic nature. However, the chemistry of these materials and the physiological processes involved in their formation are subjects requiring further investigation.

In apple wood younger than 12-15 years of age, copious quantities of wound gum or gum was formed in the vessels of the sapwood-discolored wood transition zone. Histochemical tests indicated gum was polysaccharide in nature but little else can be said of its chemical nature or structure. Gums of fruit and bark in a number of Prunus species are composed of complex polysaccharides based on glucuronic acid (Hough and Pridham, 1959; Zitko et al., 1965; cited by Rosik et al., 1968). Whether apple wound gum has a similar composition remains to be determined. Talboys (1968) suggested that two types of gum could be formed in plum wood,

and observation on gum deposits in apple wood vessels indicated that there were probably two types of apple gum - a fine and coarse textured type. Histochemical evidence indicates that apple gum contained the enzymes peroxidase and phenoloxidase, and polyphenolic material. Further investigation is required to determine whether such materials are simply included within the gum matrix or whether they are chemically linked to molecular components of gum.

Gum originated from the ray and xylem parenchyma cells. Chattaway (1949) claimed most gum was formed by ray cells and rarely by xylem parenchyma cells. In apple wood the origin of gum could be traced to xylem parenchyma cells as well as ray cells. Starch consistently disappeared from tissues where gum formation occurred and may be the material from which gum is synthesised. Further study is required to determine if hemicelluloses or wall constituents such as pectins are utilized in gum formation, or whether additional carbohydrate is translocated to the sites of gum synthesis.

The formation of gum by a ray or xylem parenchyma cell did not result in the cells immediate death. Frequently cells deep within a gum zone retained their nuclei, some starch and vital staining ability. The volume of gum manufactured by individual cells must frequently be many times the cell volume. As Chattaway (1949) suggested, gum synthesis probably represented a period of high metabolic activity for the cells following external stimulation. After gum formation, the cells appeared to die and undergo necrosis in the same manner as those cells which had not produced gum. Elucidation of the physiological processes

involved in gum formation by ray and xylem parenchyma cells provides a formidable challenge for future investigation.

With ageing, the wood xylem and ray parenchyma lost their ability to form gum. A complete loss of gum forming ability occurred at about 12-15 years of age. The decline in ability of the ray and xylem parenchyma to synthesise gum appears to be an additional aspect of the senescence of the living wood cells, normally observed across stems (see later). It is probably related to a decline in both available substrate and specific enzyme activity.

After its formation, gum gradually altered from a colorless, weakly staining, viscous material to what appeared as a hard, brittle and discolored deposit. Shrinkage of gum also occurred during maturation. These changes indicated polymerisation or loss of water from gum, although evidence favours the latter suggestion. Hough and Pridham (1959) found the hardening of plum fruit and bark gum was prevented in gum kept in a saturated atmosphere. The changes in gum after its formation may have some significance with regard to fungal penetration.

Gum was restricted in its distribution to the lumens of the structural elements of the sapwood, particularly the vessels and to a lesser extent the fibres. The occurrence of polysaccharide gum in the latter has not been previously reported. Many workers have referred to the dark colored deposits formed in xylem and ray parenchyma as wound gum (Rhoads, 1917; Higgins, 1919; Coster, 1924; Willison, 1932; Good and Nelson, 1951; Good et al., 1955; Hart, 1963). On the basis of this study, it seems necessary to consider depositions in ray and xylem parenchyma cells and the polysaccharide gum deposited in the vessels,

to be different but not necessarily completely unrelated materials.
That gum (orcinol- and Schiff's-positive material) did not occur in the
lumens of ray and xylem parenchyma, gives some credence to the suggestion
of Talboys (1968), that gum may form by the polymerisation outside the
cells, of precursors secreted through the pits.

Gum formation in the vessels beneath wounds in young wood,
effectively sealed off the underlying wood from atmospheric influence.
Evidence for this conclusion was obtained from pressure chamber studies.
As Swarbrick (1926) and Rankin (1933) suggested the initial function of
wound gum is probably a physiological one. It is a means by which the
tree may seal wounds and minimise disturbances in the wood following
wounding. This was illustrated when large branches were wounded. Dis-
coloration was more extensive in the older wood where no gum formed,
than in the younger wood.

Physical and chemical changes occurred with discoloration, which
indicated that the formation of discolored wood was associated with
mobilisation and redistribution processes. These changes included an
increase in moisture content, a decrease in pH and an increase in levels
of K, Ca and Mg, compared with sapwood. In addition, neutral solvent
extractable material and extractable phenolics decreased in discolored
tissue but this appeared to be due to changes in the nature of these
materials rather than redistribution. Moisture content, pH and levels
of K, Ca and Mg were reported to rise in discolored wood of sugar maple
(Good et al., 1955), black locust and osage orange (Hart, 1968) and Norway
spruce (Shain, 1971). Increased pH in the discolored wood of these
species may be related to increased mineral content although bacteria

could also increase the pH (Hartley et al., 1961). Most authors favour

the former explanation and Good et al. (1955) showed a relationship

between increasing mineral content and increasing alkalinity in dis-

colored sugar maple. The changes that occur in the discoloration of wood

probably have conflicting effects on pH, but in apple, as evidenced by

results under both sterile and non-sterile conditions, the net effect of

the changes resulted in a slight decline in pH. In discolored apple

wood, increases in K, Ca and Mg levels were generally proportionally

much less than those reported in discolored sapwood of sugar maple, black

locust, osage orange or Norway spruce, which may possibly explain why

pH did not increase in discolored apple sapwood.

Two physiological factors influenced the reaction of sapwood to

external stimuli -- the age of the wood and the seasonal physiological

cycle of the trees. As the former was of more significance to the host-

pathogen interaction, only seasonal effects will be considered at

present.

The host reaction to wounding appeared to be linked with the

trees seasonal cycle of physiological activity. Both the extent of

wound gum formation and discoloration were much greater beneath wounds

made in summer than wounds made in winter or autumn. Similar general

effects of season on the extent of gum formation, discoloration or wound

heartwood formation were observed by Swarbrick (1926), Wardell and Hart

(1970a) and Lyr (1967). It is of interest to note that while the rate

of gum formation was greater beneath wounds made in summer, the initial

rate of response (as judged by length of time for initial gum formation)

was similar in wounds made in all three seasons. While gum formation

was considerably reduced in winter it was not completely prevented.
This contrasted with the observation of Swarbrick (1926), who found no
gum formation beneath wounds made in winter on apple and other species
under English conditions. The Tasmanian winter conditions were
apparently not severe enough to cause a complete reduction in metabolic
activity of the xylem and ray parenchyma, or ability of the wood to
respond to the wounding stimulus.

In the present study, both the extent of discoloration and the
depth beneath wound surfaces at which gum formation commenced were
greater under summer wounds. As noted above, Lyr (1967) and Wardell and
Hart (1970a) observed more extensive discoloration from summer wounding
compared with wounds made in other seasons. The reason for this is not
clear, but it is suggested that the higher tension in the xylem water
column during summer causes a greater withdrawal of water in the vessels
from the cut surface, or a higher rate of drying of the exposed wood
occurs in summer, allowing air to penetrate further into the tissue and
thus stimulate more extensive discoloration.

Discoloration of sapwood was a host reaction of normally limited
extent which involved consistent and broadly definable physiological
processes. The host reaction occurred between healthy and fungal decayed
wood and it is probable that the interaction between host and pathogen
influenced the progress of fungal decay. In established fungal infections
there appears to be a number of aspects of the interaction between the host
(through host reaction) and pathogen which require consideration.

These are: (a) the interaction between host and pathogen in the formation of discolored wood; (b) the effect of the host reaction on the susceptibility of sapwood to decay by T. versicolor; (c) effect of variations in the host reaction on the susceptibility of wood to decay, and the pattern of fungal colonisation of living wood.

(a) The interaction between host and pathogen in the formation of discolored wood.

It was not possible to prove conclusively that T. versicolor could induce discoloration and gum formation in apple sapwood but circumstantial evidence indicates this was probably the case. The precise factors that induce discoloration of sapwood seem likely to depend on the situation and almost any deleterious agent appears likely to be able to induce sapwood discoloration. Beneath sterile wounds, one explanation is that discoloration was due to the influence of the external environment - aeration and desiccation in particular, which alter the activity of the xylem and ray parenchyma cells. Since necrosis of the cells could occur in the absence of micro-organisms, it appears that the actual process of transformation was host mediated. However, the general observation was made that discoloration was more intensive and extensive under non-sterile wounds and wounds inoculated with T. versicolor than under sterile wounds, which supports the idea that T. versicolor and other micro-organisms can induce or contribute to development of discoloration. Similar observations were made by Grosclaude (1966b) and Shigo (1967a).

In established fungal infections physical factors are likely to be less important than chemical factors in inducing discoloration. Discoloration and death of cells could be induced by fungal enzymes or metabolites, wood break-down products, host products (e.g. hormones) or the activities of other organisms in advance of decay. However, which factor(s) contribute most to the induction of discoloration remains to be elucidated.

Evidence that some translocatable factor(s) was produced (originating from the host, pathogen or otherwise), capable of inducing discoloration in advance of decay comes from an examination of the pattern of discoloration around T. versicolor infections in old and young wood. Discoloration was always more extensive in longitudinal than lateral extent which could indicate that some material detrimental to the wood cells was produced and acted at a distance. In young wood it is possible that gum formation could have limited translocation or diffusion of discoloration inducing products. However, this is an aspect relevant to the postulation considered previously that gum has an initial physiological function.

(b) Effect of host reaction on the susceptibility of sapwood to decay by T. versicolor.

In vitro decay tests indicated that discolored sapwood was less susceptible to decay by T. versicolor than normal sapwood. This suggests discoloration of the wood functioned as an active host resistance mechanism to fungal colonisation and decay in vivo. However, care is required in the extrapolation of this result to the in vivo situation because of the unknown effects of the interaction between

T. versicolor and other micro-organisms present in the discolored wood in advance of decay.

It seems probable that the host reaction increases the decay resistance of sapwood in vivo, although it was not possible to investigate the interaction of T. versicolor with other micro-organisms present around decay zones in apple limbs. A small number of bacterial species could usually be isolated from discolored wood in advance of the fungus. Good et al. (1968) reported that bacteria and Fungi Imperfecti probably destroyed the inhibitory effects of the reaction of maple wood in advance of Fomes igniarius. In maple, the host reaction was associated with a sharp rise in pH and under these circumstances F. igniarius appeared as a secondary coloniser, dependent on the activity of the other organisms for its success as a pathogen. That the susceptibility of wounds in young apple wood to invasion by T. versicolor decreased rapidly with ageing of the wounds, favours the view that T. versicolor is probably a primary coloniser, and its success as a pathogen does not depend to any significant degree on its association with other micro-organisms. It is also possible that micro-organisms may contribute to the resistance of the sapwood to fungal invasion as suggested in the case of Fomes annosus in Norway spruce (Shain, 1971).

A number of changes involved in the discoloration of the wood, seem likely to contribute to the increased decay resistance of dis-colored wood. One major effect of the host reaction would be to deprive the fungus of readily assimilated food material, particularly carbo-hydrates and nitrogenous compounds. Cytoplasmic and reserve food materials underwent conversion and redistribution with discoloration.

Analyses confirmed that discolored wood was very low in soluble sugars, starch and nitrogen, but levels of hemicelluloses appeared unaffected by discoloration of the wood. Results from in vitro studies suggest that the decay resistance of discolored wood is probably increased by the loss of readily utilised food material from the cells. Merrill and Cowling (1966b) and Levi and Cowling (1968) showed that sapwood of Populus grandidentata and Quercus falcata of low endogenous nitrogen status (and probably also low carbohydrate status, although this was not checked by the authors) was more resistant to decay than wood of higher nitrogen content. Hot water extraction of pine and spruce sapwood increased its decay resistance compared with unextracted controls (Peterson and Cowling, 1964) and they suggested the increase in decay resistance was due to the removal of low molecular weight nitrogen and carbohydrate compounds from the wood.

The most probable source of decay resistance in discolored sapwood is the presence of fungal inhibitory phenolic compounds in cell walls and the lumens of ray and xylem parenchyma cells. Phenolic compounds are the major source of decay resistance in heartwood (Scheffer and Cowling, 1966) and discolored sapwood normally contains phenolic compounds similar to those in the heartwood of the same species (Hillis, 1968). Discolored apple sapwood contained high and low molecular weight phenolic material and preliminary chromatographic evidence indicates the latter compoundswere probably of the quercetin-quercitrin type and phloridzin. Much of the high molecular weight material appeared to be unextractable by the methods employed. The small amounts of low molecular weight phenolics in discolored wood appear unlikely

to be inhibitory to T. versicolor, as the fungus can metabolise quercitrin and rutin (Pickard and Westlake, 1969) and a range of phenolic glycosides (Lafuse, 1937). In the present study, it was not possible to determine whether phenolics extracted from healthy or discolored apple sapwood were inhibitory to T. versicolor. Evidence obtained by Somers and Harrison (1967), Palct (1967) and Harrison and Clare (1970) indicated that high molecular weight phenolic and tannin material present in discolored sapwood and heartwood of apricot was inhibitory to Verticillium dahliae and T. versicolor, in vitro. Any assessment of the inhibitory nature of material deposited in the cells in dis-colored apple wood is complicated by the largely unextractable form of the material.

The presence of gum in the vessels and fibres in discolored wood younger than 12-15 years of age appears likely to impede fungal penetration and decay of the wood. Brooks and Storey (1923) used the term 'gum barrier' to describe dense areas of gum formation in plum wood that were capable of limiting the spread of Stereum purpureum. This terminology was not adopted in the present study because, although the gum produced in apple wood around fungal infections formed a definable zone, it did not act as an absolute barrier to the spread of T. versicolor. The gum zone was not a static but a dynamic feature - gum was being formed on the outside of the zone as it was being destroyed on the inside by the fungus.

The presence of gum in the vessels and fibres could adversely affect colonisation and decay by the fungus in a number of ways:

(i) By presenting the fungus with a large volume of material it must
     metabolise before it can attack the wood.

(ii) Gum could physically or chemically protect cell wall material from
     fungal attack.

(iii) Gum may disrupt the translocation/diffusion of gases and liquids
      away from sites of fungal activity leading to a possible build-up
      of fungal inhibitory materials or aeration or moisture content may
      not be optimal for fungal activity in gummed wood. Evidence has
      been presented to illustrate the highly impermeable nature of gummed
      wood.

(iv) The actual physical or chemical nature of the gum or presence of
     extraneous polyphenolic material in the gum may inhibit the fungus.

The magnitude of other physio-chemical changes that occurred in
discolored wood seems unlikely to markedly affect the resistance/
susceptibility of wood to decay in vivo. The small decrease in pH would
appear to favour T. versicolor, while the rise in levels of mineral
elements is unlikely to affect the fungus. The rise in water content of
discolored zones may be unfavourable to decay but wood rotting fungi are
generally favoured by moisture contents above fibre saturation point
(Merrill, 1970) and T. versicolor can grow at very low oxygen tensions
(Scheffer and Livingston, 1937).

> (c) Effect of variations in the host reaction on the susceptibility
>   of wood to decay and the pattern of fungal colonisation of
>   living wood.

The susceptibility of the wood of apple trees to decay by T.
versicolor varies with the age of the wood. Young wood (arbitrarily
defined as wood less than 10 years of age) was less susceptible to decay

than old wood. This conclusion is based on two observations. Firstly, natural infections seldom occurred in limbs younger than 10 years of age (although it is possible to establish infections in young wood by artificial inoculations) and secondly, in infected limbs (either naturally or artificially infected) the greatest fungal penetration and decay was in the oldest wood to which the fungus had access. Additional evidence to support the conclusion was the very low rates of penetration by T. versicolor observed in young wood. While no comparable data is available for old limbs, rates of penetration and decay must certainly be many times higher than those observed in young wood, if only to account for the amount of decay observed in field grown trees.

Under Tasmanian conditions apple trees do not form heartwood until they are at least 40-50 years of age. Most large limbs therefore, contain only sapwood. There was however, a radial decrease in vital staining and levels of carbohydrates, nitrogen and phosphorus from the cambium to the pith. This indicates that the ageing or senescence of the living wood cells is accompanied by a gradual decline of their physio- logical activity (Ziegler, 1967). Similar radial variations in a range of cellular properties across tree stems were reported by Frey-Wyssling and Bosshard (1959), Priestley (1962b), Merrill and Cowling (1966a) and Ziegler (1967). In trees which form heartwood, death of the cells occurs at the sapwood-heartwood transition zone. As well as a decline in the metabolic activity of wood cells, ageing also results in a change in the functional nature of the wood. In most trees water conduction appears to be restricted to the outermost annual rings of sapwood and Ziegler (1967) suggested the differentiation of sapwood into the outer

sapwood, which can conduct water, and the inner portion of the sapwood which has a purely food storage function. The results of Darbyshire (1967) for P^{32} and dye injections into apple trees, indicates most conduction is in the outer sapwood, although the results cannot be regarded as conclusive due to problems of lateral distribution.

The ability of the host to react to external stimuli also declined with ageing of the wood. The most obvious feature of this decline was the lack of wound gum synthesis in wood greater than 12-15 years of age. In addition, smaller amounts of extraneous materials were deposited in the discolored ray and xylem parenchyma cells in old wood compared with young wood. A number of authors have observed that young wood responds more intensely to external stimuli than older wood (Rhoads, 1917; Swarbrick, 1926; Brooks and Brenchley, 1931; Good et al., 1955).

The evidence from a study of host reaction per se, favours the hypothesis proposed by Shain (1967), that the resistance of sapwood to attack and decay is dependent on the vitality of the living cells and their ability to elaborate an effective inhibitory barrier against the fungus. It is proposed that the general non-susceptibility of young apple sapwood to T. versicolor, is associated with the rapid and continuing formation of wound gum and the greater amounts of extraneous material deposited in the xylem and ray parenchyma cells around infections and wounds in these tissues. With ageing of the wood there is a synchronous decline in cellular properties, although properties are adjusted to a level to maintain the integrity of the cells. In terms of the trees physiology the cells are maintained at an adequate level. It seems they have little or no physiological stress placed upon

them, other than as a storage tissue and function as efficiently as required by the tree. In a stress situation, such as protecting the wood from fungal invasion, the cells in older wood do not have sufficient vitality or synthetic ability to produce a completely efficient fungistatic host reaction. The resistance of plum sapwood to invasion by Stereum purpureum, and pine and spruce sapwood to Fomes annosus has been associated with the vital nature of the host sapwoods and their ability to form an effective inhibitory barrier against these fungi (Brooks and Storey, 1923; Brooks and Moore, 1926; Brooks and Brenchley, 1931; Brooks, 1936; Shain, 1967, 1971).

Two points of circumstantial evidence suggest that the physiological vitality of young wood determined its susceptibility to fungal colonisation and decay. Firstly, living limb sections incubated in an atmosphere at 100% R.H. were rapidly colonised by the fungus without wound gum formation in the wood. Secondly, in a number of T. versicolor inoculations in the P experiment there appeared to be an interaction between the host and micro-organisms other than T. versicolor, which altered the host reaction. The wood was killed and discolored in advance of the fungus and gum formation was prevented in wood which normally formed abundant gum. The maximum length of fungal penetration in these inoculations was normally several centimetres greater than the treatment average. While this effect cannot be positively attributed to lack of gum formation in the wood, it appeared the effectiveness of the host reaction to prevent fungal colonisation and decay had been reduced.

It is yet to be determined whether the formation of discolored wood, around established infections of T. versicolor is continuous

throughout the year. In young wood the host reaction to wounding appeared linked to the trees seasonal cycle of physiological activity. That a zone of discolored wood was always present between healthy and infected sapwood in all seasons, indicated that if variations in the intensity or rate of host reaction did occur with season, then they do not have any major effect on the balance between host and pathogen. Likewise, seasonal conditions may affect the activity of the fungus in the wood.

From an examination of the host reaction per se, it is also possible to explain the pattern of fungal colonisation and decay observed in living wood. Normally within a limb, maximum longitudinal penetration occurred in the vessels (path of least inhibition), followed by radial spread into the rays (ray cells being proposed as the site of greatest fungal inhibition with high levels of polyphenols), vessels and fibres. The extent of radial spread was small compared with the extent of longitudinal movement. Likewise, greatest colonisation and decay occurred in the oldest xylem tissue to which the fungus had access and in which the host reaction was least effective in inhibiting the fungus. The change in susceptibility of wood to decay with ageing therefore leads to the V-shaped infection fronts observed in limbs and the tendency for rots to be located in the centre of limbs. Brooks and Brenchley (1931) came to a similar conclusion concerning the pattern of colonisation of Stereum purpureum in plum and numerous authors have observed that sapwood rots are most extensive in the oldest wood (Good and Nelson, 1951; Good et al., 1955; Shigo, 1965; Shigo and Sharon, 1968).

The colonisation of living wood in vivo, contrasts with the pattern of wood colonisation frequently observed in vitro with T. versicolor and other wood rotting fungi. In vitro, medullary ray cells frequently serve as an initial site of penetration and colonisation (Bayliss, 1908; Nutman, 1929; Greaves and Levy, 1965).

Wade (1968) suggested that the pith column in apple limbs could serve as a means of longitudinal penetration of limbs by T. versicolor. This idea was dependent on the hypothesis that the thin walled pith parenchyma offered a path of least physical resistance and high nutrient availability. Within an infected limb, fungal penetration of the pith could equal or exceed penetration in the inner secondary xylem. However, in the majority of natural and artificial infections the maximum extent of penetration occurred in the xylem tissue and colonisation of limbs did not appear dependent on pith penetration.

Pruning wounds serve as the means of entry of T. versicolor into the host sapwood. The susceptibility to infection of wounds made in young apple wood in summer, autumn and winter, both in terms of longitudinal hyphal penetration and amount of hyphae in the wood, decreased rapidly with ageing of the exposed wood. Wounds 15 and 30 days old were much less susceptible to colonisation than fresh wounds, although the additional ageing from 15-30 days appeared to have little effect on susceptibility. It seems possible to extrapolate this result to large wounds exposing a greater range of wood ages, because the same cellular changes occurred in old wood, although they were more restricted.

It therefore would seem unlikely that natural infection by spores could occur in wounds that had aged for any length of time.

An artificial method of inoculation was used to establish T. versicolor infections (compared with natural spore infection). Results obtained on changes in the susceptibility of wounds to infection were thought to provide a reasonable estimate of the field situation because the method of inoculations appeared highly favourable for fungal infection. In young wood, the method of inoculation modified the host reaction to wounding. It delayed the formation of gum and discoloration of the cells beneath wound surfaces, it increased the depth below the wound surface at which gum formed and possibly increased the amount of gum formed. The net effect of these changes appeared to be favourable to the establishment of fungal infection. The fungus was allowed easier initial penetration of a greater volume of tissue and would be less subject to desiccation. By delaying changes in the xylem and ray parenchyma cells, the method of inoculation probably provided the fungus with a better nutritional environment for establishment. In addition, it was possible to establish infections in wood of all ages in all seasons using the method of inoculation.

The decline in susceptibility of wounds was paralleled by discoloration and gum formation in the wood beneath the wound surface. Cellular changes probably commenced immediately after wounding, although observations were only made after 15 and 30 days. By 30 days a consistent pattern of changes was observable. A narrow zone of ray, xylem and pith parenchyma cells at the wound surface retained their starch in its granular form. Below these surface cells was an irregular

zone of cells which showed _in situ_ aggregation and disorganisation of starch granules. This zone graded into the normal sapwood-discolored wood transition zone where cells lost their starch by hydrolysis and gum was formed in the vessels. The extent of the various zones depended on the season. In view of the changes that occurred in cells beneath wounds, the decline in favourability of wounds for colonisation by _T. versicolor_ could be due to:-

(i)   Desiccation of surface tissue or loss of nutrients necessary as a food base for fungal spores to germinate and establish an initial infection. Such a loss could occur by leaching, utilisation by other organisms or conversion by the host. Manion and French (1969) found glucose was necessary for the germination of basidio-spores of _Fomes igniarius_. Spore germination decreased as the age of wood increased across stems (less water soluble carbo-hydrates), and as the age of wound increased and water soluble carbohydrates disappeared from the wound surface.

(ii) In young wood, formation of a gum zone close to the wound surface could make the fungus more susceptible to the external physical environment by preventing water movement from within the wood.

(iii) Formation of inhibitory materials by the host or colonisation by other micro-organisms inhibitory to _T. versicolor_. In this study, there was generally a low level of colonisation of wounds by fungi (in terms of the amount of hyphae) and any inhibition by micro-organisms would probably be due to bacteria. The complexity of infection court changes indicates that the decline in suscepti-bility of wounds to infection with age, could involve a combination of these and other factors (Merrill, 1970).

The limited wound response in old wood may possibly explain the greater facility with which wounds in old wood appeared to become infected with T. versicolor, compared with wounds in young wood. A number of the large branch stubs used to study host reaction in relation to the age of wood became naturally infected with T. versicolor. No natural T. versicolor infections were observed on any of the un-inoculated wounds used in experiments in this study (normally in wood up to 5-years-old).

Wounds in young wood appeared to be most susceptible to colonisation and penetration in summer. This was possibly related to the greater depth below the wound surface at which gum formed in summer, a more favourable nutritional environment for establishment, or it could indicate that fungal activity was favoured by the higher summer temperatures. Winter appeared to be the normal season of infection in the field. Orchard pruning is undertaken in winter and from observation on sporophore development, autumn-winter is probably the period of maximum inoculum availability.

The apparent rapid decline in the susceptibility of wounds to infection is of significance to the practical control of T. versicolor. Modification of infections courts is one method of controlling diseases such as T. versicolor (Merrill, 1970). The results indicate the need for short term protection of wounds and particularly those wounds exposing old wood, against infection by the fungus.

Experiments were undertaken to study susceptibility of young apple trees to $\underline{T}$. versicolor in relation to nitrogen and phosphorus nutrition, and to water stress. Nitrogen nutrition and water stress are of general importance to tree health in the field, and tree susceptibility in relation to these factors is of some practical significance. Phosphorus nutrition may prove to be of significance to tree susceptibility in the field. Field responses to phosphatic fertilisers have not been reported in Tasmania. Recent evidence however, indicates that in some field situations in Tasmania, imbalances in fertilization may lead to low tree phosphorus levels (Richards, private communication).

The growth and nutritional status of experimental trees were strongly influenced by the treatments imposed. Nitrogen and phosphorus deficiency and water stress treatments reduced tree growth and altered the concentrations of mineral elements and carbohydrates in the bark and wood of the trees. One effect not previously reported by other authors, was that water stress increased the levels of phosphorus in the bark and wood of trees in the N x W experiment. The effect of water stress leading to increased tissue nitrogen concentration is well known, but the effect observed with phosphorus was further complicated in that leaf phosphorus levels were significantly less in water stressed trees than in trees watered normally. The variation in phosphorus level between W_0 and W_1 treatments may indicate that the difference in phosphorus uptake between W_0 and W_1 trees was less than the difference in dry matter production between W_0 and W_1 trees, leading to increased phosphorus levels in the storage tissue. Alternatively, it is possible that water stress leads to a greater proportion of the absorbed

phosphorus being retained in the wood and bark due to disruption of translocation to the leaves. This is supported by the fact that levels of K, Ca and Mg were reduced in leaves under water stress. The supply of calcium to leaves and fruits is dependent on xylem transport and when this is restricted or eliminated calcium content is affected accordingly (Wiersum, 1966). Withdrawal of water from leaves may also occur under water stress and this could enhance phloem movement of phosphorus out of leaves (West et al., 1970).

In established T. versicolor infections, colonisation and decay of the living sapwood depends on the interaction between the host (through host reaction) and the fungus. When considering the suscepti-bility of living apple sapwood to decay by T. versicolor, it is necessary to consider the nutritional environment of the fungus and the host inhibitory environment. The nutritional environment of the fungus is the cell wall constituents of the wood and the material deposited in the wood cells by the discoloration of the wood. From studies of the nature of the host reaction and the spatial relationship between host and pathogen, it appears that there is no direct relationship between the nutritional status of the healthy living wood and its susceptibility to decay as postulated by Darbyshire (1967) and Darbyshire et al. (1969). Rather, nutrition probably has an indirect effect on the susceptibility of wood to decay through its effect on host reaction or host ability to react. Thus, because of the host reaction in the sapwood the fungus did not have access to easily assimilated substrate such as soluble sugars and starch. However, the nutritional status and physiological condition of xylem and ray parenchyma influences the host reaction.

One example of this is the effect of the age of the wood on the host reaction.

In the N x W experiment, measurements of both papery bark and hyphal penetration indicated trees of the normal and high nitrogen treatments were more susceptible to T. versicolor than trees of the low nitrogen treatment. Evidence previously discussed indicated that the length of fungal penetration (decay) is the most satisfactory means of measuring the susceptibility of trees to T. versicolor. The measurement of hyphal penetration may be regarded as an estimate of the amount of wood decayed. Decay of the wood always appeared to be a natural consequence of penetration by T. versicolor, no matter what the physiological condition of the host. In addition, the diameter of all limbs inoculated was the same, and microscopic examination indicated the degradation of the wood fabric by the fungus had proceeded to a similar degree in all treatments. Thus, trees of the nitrogen deficiency treatment were less susceptible to decay by T. versicolor than trees of the normal and high nitrogen treatments.

The effect of nitrogen deficiency on susceptibility of limbs to penetration and decay appeared to be a nitrogen effect per se, although other wood nutrient levels were altered by the treatments. There are two points of evidence for this conclusion. Firstly, while there were differences in wood phosphorus levels (and as judged by leaf analyses also in K, Ca and Mg levels) between nitrogen treatments and between watering treatments, these latter differences had no effect on susceptibility. Secondly, available carbohydrates in the wood were decreased under nitrogen deficiency and water stress treatments but there

was no difference in susceptibility of trees to $\underline{T}$. $\underline{versicolor}$ between W_0 and W_1 treatments. While the possibility exists that some other associated physiological or nutritional condition was controlling susceptibility to penetration and decay, the evidence indicates that the nitrogen status of the wood was the major factor.

The influence of low nitrogen content of the wood on its susceptibility to decay may be explained in two ways. Firstly, the host reaction could more effectively limit fungal penetration and decay at low nitrogen levels and secondly, that the effect of wood nitrogen level was a nutritional effect i.e. the nitrogen content of discolored wood was too low to support maximum growth and penetration by the fungus. The fact that there was no measurable effect of nitrogen status on the ability of the wood to form gum or the intensity of discoloration of the wood, indicates the effect of nitrogen status was probably a nutritional effect. The differences in fungal penetration in limbs of trees at the N_1 and N_2 levels, although not statistically significant, suggests that high wood nitrogen levels may have an inhibitory effect on wood decay. The effect observed could have been a toxicity effect due to imbalances in nitrogen metabolism caused by the high level of nitrogen supply or alternatively high nitrogen levels may have stimulated the host reaction. Further research is required to test whether nitrogen content of the wood has a threshold value for maximum decay susceptibility. Because of the limited number of nitrogen levels used in this experiment, it is not possible to determine whether the control level (N_1) was optimal or limiting for decay.

The present study showed that the endogenous nitrogen status of the wood is probably one important factor in the decay susceptibility of living wood _in vivo_. Thus, wood of the same age in different trees will be most susceptible to decay in the tree whose wood has the highest nitrogen status, provided other conditions are not affecting the decay susceptibility. Merrill and Cowling (1966b) and Levi and Cowling (1968) demonstrated the probable importance of the endogenous nitrogen content of the wood on its susceptibility to decay _in vitro_. Sapwood of high nitrogen content was more susceptible to decay than wood of low nitrogen content. While their results were well correlated with observed differences in the nitrogen status of the wood, the endogenous carbohydrate status of the wood probably also had a significant effect on its decay resistance. Carbohydrates would be expected to show a similar change in level as the nitrogen content of the wood [not the reverse as proposed by Darbyshire et al. (1969)], since the wood was taken from different positions within a stem and from the same trees in different seasons. Cowling et al. (1969) found no differences in the decay susceptibility of wood from nitrogen fertilised or unfertilised trees of _Pinus silvestris_ and _Picea abies_. However, the nitrogen content of the wood had not been affected by the treatments applied.

Within single tree stems or limbs the oldest sapwood, which has the lowest nitrogen content (Merrill and Cowling, 1966a; present study), is the most susceptible to decay by fungi such as _T. versicolor_. One explanation for this situation is that in the decay of sapwood of living trees, the nitrogen content of the wood is not the limiting factor. The limiting factor is the ability of the host cells to

elaborate an inhibitory environment which will limit the fungus. The nitrogen content in old apple stem wood is very low (0.07-0.10% compared with 0.25% in nitrogen deficient 3-year-old wood in N x W experiment) but the host resistance has declined to such an extent, that the overall balance is much in favour of the fungus, leading to extensive wood decay. While the nitrogen content of the wood declined under artificially induced nitrogen deficiency, there was no indication that the effectiveness of the host's inhibitory environment declined. Recent evidence indicates that wood rotting fungi are especially efficient in their nitrogen metabolism and possibly re-utilise mycelial nitrogen (Levi et al., 1968). This adaptive feature wound seem the most likely explanation for the ability of wood decay fungi to destroy large amounts of wood of extremely low nitrogen content.

Thus, the susceptibility of wood to decay in living trees is probably determined by the balance between the inhibitory environment of the host and the pathogenic ability of the fungus, but factors such as nitrogen content of the wood may affect susceptibility to decay. Theoretically, if trees have low wood nitrogen content but high host resistance, their wood will be less susceptible to decay than wood in trees with a low nitrogen content but low host resistance. Likewise trees with high wood nitrogen but low host resistance should be most susceptible to decay. Therefore, even in old stems it is possible that the nitrogen content of the wood may be limiting decay.

Neither low nitrogen nor water stress treatments affected the host reaction or at least the host gum forming ability. Gum is predominantly polysaccharide and it is probable that treatments which alter

carbohydrate levels could affect the amounts of gum formed. The depletion of carbohydrate reserves was apparently not sufficient to significantly alter the balance between host and pathogen. Internal water stress could possibly affect gum formation in the wood during periods of low moisture availability. Unfortunately, inoculations were made in winter and a well established gum zone was present before severe water stress was induced in the following growing season. Such a barrier would help to insulate the fungus from the direct effects of water stress, and possibly explains the lack of effect of water stress in this experiment.

The depletion of wood phosphorus reserves was slower than expected, and only a moderately low phosphorus status was achieved in the time available for the P experiment. At the end of the second growing season, levels in the 2-year-old wood were reduced to 227 ppm in the P_0 trees compared with 390 ppm in the 2-year-old wood of control trees. The slow depletion of reserves is probably explained by the low phosphorus requirement of apple trees. Batjer and Degman (1940) reported 1-year-old apple trees grew normally on nutrient solutions containing 4 ppm phosphorus and deficiency symptoms only developed when phosphorus was completely lacking. In the present experiment growth was reduced and moderate leaf symptoms of phosphorus deficiency were visible in the second and third growing seasons on trees in the P_0 experiment. Leaves showing phosphorus deficiency symptoms had a phosphorus content of 0.07-0.08%. Davis (1930) and Mochizuki and Hanada (1958) reported similar levels in leaves showing phosphorus deficiency

symptoms. High initial phosphorus status of trees could also have contributed to the slow depletion of reserves.

Phosphorus treatment did not influence the susceptibility of wood to fungal penetration and decay, or affect the host reaction although papery bark formation indicated P_0 trees were more susceptible to T. versicolor than control trees. This result indicates that, neither the phosphorus depletion nor the other nutritional changes observed in the wood, were great enough to alter the balance between the host and the pathogen. Apparently only under severe phosphorus deficiency, such as achieved by Wade (1968), did the nutritional environment of the wood become particularly favourable or host resistance less effective, leading to increased susceptibility to fungal attack.

Compared with the levels of wood phosphorus reported by Wade (1968), the levels in this experiment can only be considered moderately deficient. Wade found 4-year-old inoculated limbs had a mean phosphorus level of 55 ppm, compared with 180 ppm in the controls. In the present experiment the level in 2-year-old wood at inoculation was 227 ppm compared with 390 ppm in the controls. Wade's result was obtained from wood sampled in summer when levels are a minimum. Wade however used 4-year-old wood (cf. with 2-year-old wood), and his trees were grown under phosphorus deficiency conditions for a longer period than in the present experiment. It is also possible that the phosphorus deficiency condition observed by Wade was aggravated by the high K/Mg ratio in his phosphorus deficient trees. Magnesium may be a carrier for phosphorus (Truog et al., 1947) and magnesium is known to

activate enzymes reactions involving phosphorus (Bear et al., 1951).
Thus, the discrepancy between results in this study and those of Wade
(1968) was probably due to the much more severe phosphorus deficiency
condition induced by the experimental treatments used by that author.

In both the N x W experiments significant nutritional differences
were induced in the wood by the experimental treatments. None of these
differences had a measurable effect on the host reaction. This
indicates that the treatments were not effective in influencing the key
processes involved in the host reaction or that the young ray and xylem
parenchyma cells had such a high level of metabolic activity that they
could tolerate big nutritional changes without obvious effect on the
host reaction. That it was not possible to induce differences in
susceptibility in young trees by water stress or moderate phosphorus
deficiency, suggests that in the field ageing is probably the dominant
factor altering the physiological activity of the wood and influencing
susceptibility of trees to T. versicolor.

From results in this study, it is possible to propose an
explanation for the results of Wade (1968). The phosphorus content of
the sapwood of the large apple limbs used to study host reaction in
relation to wood age showed a decline from about 180 ppm in the wood
adjacent to the cambium to a level of 70-90 ppm in the oldest wood.
The level in the old wood compared closely with the phosphorus content
of 4-year-old phosphorus deficient wood reported by Wade (1968).
The levels present in old wood naturally, and inducable in young wood,
show that a high proportion of the phosphorus initially accumulated in
the wood can be translocated out of the wood. The results also

indicate that it is possible under conditions of phosphorus deficiency, to achieve a level of wood phosphorus that is normally reached in the wood by ageing. Phosphorus is intimately involved in structure and energy balance of living cells as a component of membranes,nucleic acids, phospholipids ATP and the coenzymes NAD and NADP (Hageman, 1969). Any decline in phosphorus content of cells seems likely to have a drastic effect on cellular functioning,and phosphorus deficiency is known to cause anatomical variations and premature senescence in a number of plants (Sommer, 1936; Lyon and Garcia, 1944). Under conditions of phosphorus deficiency the translocation of phosphorus from old to young tissue is likely to hasten the senescence of the older tissue. It is proposed therefore, that severe phosphorus deficiency in young wood could affect its susceptibility to decay in a manner synonymous with ageing in old wood. It may cause a decline in cell vitality and synthetic ability such that wood is not capable of elaborating an effective inhibitory barrier. Severe phosphorus deficiency could inhibit the formation of wound gum, the synthesis of phenolics and disrupt mobilisation and re-distribution processes involved in discoloration. Indirect evidence that the effect of phosphorus deficiency on susceptibility observed by Wade (1968) was not connected with the vital nature of the wood and not fungal nutrition, is the generally low mineral requirements of wood rotting fungi (Merrill, 1970) and the lack of any reported effect of phosphorus on wood decay _in vitro_.

Other factors which influence the susceptibility of field grown trees to _T_. _versicolor_ may act in a similar manner to that

proposed for nitrogen and severe phosphorus deficiency. While the age of trees is probably the most generally important factor increasing their susceptibility to T. versicolor, other nutritional, environmental or cultural practices which alter the host resistance will alter susceptibility. These may include high or low vigour inducing treatments. Thus, a higher incidence of T. versicolor infection has been associated with the decline in tree vigour due to overcropping, drought, and root damage (Doepel, 1965). It is possible to envisage these factors altering host resistance by an effect on the vitality of the wood or on the amounts of reserve materials which could be utilised in the host reaction. In Tasmania, one of the highest incidences of T. versicolor results from the re-working of old trees. In this situation there are a large number of infection courts exposing old wood and in addition, the severe imbalance between the roots and aerial portion of the tree normally causes excessive vigour in the new scions. This could lead to low host resistance through depletion of reserve food materials in the wood.

Conclusions

1. Trametes versicolor is a facultative parasite which can attack the sapwood of apple trees.

2. Papery bark is a characteristic but non-specific bark symptom observed on limbs infected with T. versicolor.

3. Apple sapwood reacts to wounding and fungal invasion in a non-specific and dynamic manner by the formation of discolored wood.

Discoloration involves the disappearance of starch from and the necrosis of xylem and ray parenchyma cells, with the probable accumulation of oxidised phenolics in the cells. In wood less than 12-15 years of age polysaccharide wound gum is produced by the xylem and ray parenchyma and deposited in the vessels in discolored wood.

4. Evidence indicates that the host reaction per se makes sapwood less susceptible to decay by T. versicolor. The resistance of young sapwood to decay appears to be due to the vitality and ability of the xylem and ray parenchyma cells to elaborate an efficient inhibitory environment against T. versicolor, particularly by wound gum formation and the deposition of large quantities of extraneous materials in the ray and xylem parenchyma cells.

5. It is proposed that the increasing susceptibility of wood to decay with ageing is related to the natural decline in cellular properties and synthetic ability of ray and xylem parenchyma and the consequent inability of the wood to elaborate an effective inhibitory barrier against the fungus.

6. Treatments (apart from ageing) which alter susceptibility may do so by their effect on the fungal nutritional environment in the wood (as is probably the case with nitrogen), and by their direct or indirect effects on the vital nature of the ray and xylem parenchyma cells and consequently on host reaction. These effects result in the alteration of the balance between host and pathogen which may lead to increased or decreased susceptibility to T. versicolor.

BIBLIOGRAPHY

Anon (1956). Wood rot of fruit trees. N.S.W. agric. Gaz., Plant disease leaflet No.91.

Bailey, R.W. (1958). The reaction of pentoses with anthrone. Biochem. J. 68: 669-672.

Baker, C.E. (1931). Water conductivity in the apple tree as affected by pruning and drought. Ph.D. thesis, University of Illinois.

Bakshi, B.K. and Singh, S. (1970). Heart rots in trees. Int. Rev. Forest. Res. 3: 197-251.

Barčukova, M.V. (1967). 'Wound reaction' of wood parenchyma cells of certain broad leaved species. For. Abstr. 29: No.2905.

Batjer, L.P. and Degman, E.S. (1940). Effects of various amounts of nitrogen, potassium and phosphorus on growth and assimilation in young apple trees. J. agric. Res. 60: 101-116.

Baxter, P. (1957a). Fertilizer trials on apple and pear orchards in Southern Victoria. Part I. J. Vict. Dep. Agric. 55: 351-359.

Baxter, P. (1957b). Fertilizer trials on apple and pear orchards in Southern Victoria. J. Vict. Dep. Agric. 55: 487-497.

Bayliss, W.M. (1908). The biology of Polystictus versicolor. J. econ. Biol. 3: 1-24.

Bear, F.E., Prince, A.L., Toth, S.J. and Purvis, E.R. (1951). Magnesium in plants and soils. New Jers. agric. Exp. Sta. Bull. 760: 3-24.

Beckman, C.H. (1966). Cell irritability and localisation of vascular infections in plants. Phytopathology 56: 821-824.

Beever, D.J. (1970). The relationship between nutrients in extracted xylem sap and the susceptibility of fruit trees to silver-leaf disease caused by Stereum purpureum (Pers.) Fr. Ann. appl. Biol. 65: 85-92.

Birmingham, W.A. (1936). Wood rot and limb blight of fruit trees. N.S.W. agric. Gaz. 47: 507-511.

Blumer, S. and Nuesch, J. (1962). Polyporus versicolor (L.) Fr. als Schädling an Kirschbäumen. Phytopath. Z. 45: 301-311.

Boggie, R. (1970). Moisture characteristics of some sand-peat mixtures. Scient. Hort. 22: 87-91.

Brooks, F.T. (1925). _Polyporus adustus_ (Willd.) Fr. as a wound parasite
of apple trees. Trans. Br. mycol. Soc. 10: 225-226.

Brooks, F.T. (1936). The resistance of trees to ligneous fungi. 3^e Congr.
intern. Pathologie comparee Athenes, t. 1: 135-142.

Brooks, F.T. and Storey, H.H. (1923). Silver-leaf disease - IV.
J. Pom. hort. Sci. 3: 117-141.

Brooks, F.T. and Moore, W.C. (1926). Silver-leaf disease - V. J. Pom.
hort. Sci. 5: 61-97.

Brooks, F.T. and Brenchley, G.H. (1931). Silver-leaf disease - VI.
J. Pom. hort. Sci. 9: 1-29.

Büsgen M. and Münch, E. (1929). Structure and life of forest trees.
(Translation by T. Thomson.) Chapman and Hall, Ltd., London.

Butler, C. (1911). A study of gummosis in Prunus and Citrus with
observations on squamosis and exanthema of the citrus.
Ann. Bot. 25: 107-153.

Cartwright, K. St. G. (1929). A satisfactory method of staining fungal
mycelium in wood sections. Ann. Bot. 43: 412-413.

Cartwright, K. St. G. and Findlay, W.P.K. (1958). Decay of timber and
its prevention. 2nd Ed. Her Majesty's Stationary Office,
London.

Chattaway, Margaret M. (1949). The development of tyloses and secretion
of gum in heartwood formation. Aust. J. Sci. Res. (Ser. B)
2: 227-240.

Cheung, D.S.M. (1969). Comparative analyses of antigenic and enzymatic
characteristics of the genus Trametes (Polyporaceae).
Ph.D. thesis, University of Tasmania.

Committee on Nomenclature (1964). Multilingual glossary of terms used in
wood anatomy. 186pp. Comm. Nomen. Inter. Assoc. Wood
Anatomists.

Conn, H.J. (1961). Biological Stains. 7th Ed. The Williams and Wilkins
Co., Baltimore.

Coster, C. (1924). Die physiologische und pathologische Kernholzbildung
bei Tectona grandis L.F. nebst Bemerkungen uber die
Bildung des Wundholzgummis. Ann. Jard. Bot. Buitenz. 34:
1-16.

Côté, W.A. Jr. (1967). Wood ultrastructure. An atlas of electron micrographs. University of Washington Press, Seattle.

Coutts, M.P. (1969). The formation of polyphenols in small blocks of Pinus radiata sapwood with and without the fungal symbiont of Sirex. Aust. For. Res. 4: 29-34.

Cowling, E.B., Dillner, B. and Rydholm, S. (1969). Comparative decay susceptibility of sapwood in nitrogen-fertilized and non-fertilized stands of Norway spruce and Scots pine. Phytopathology 59: 1022.

Cowling, E.B. and Merrill, W. (1966). Nitrogen in wood and its role in wood deterioration. Can. J. Bot. 44: 1533-1544.

Cunningham, G.H. (1965). Polyporaceae of New Zealand. N.Z. Dep. Sci. industr. Res. Bull. 164.

Da Costa, E.W.B. and Rudman, P. (1958). The causes of natural durability in timber. I. The role of toxic extractives in the resistance of tallowwood (Eucalyptus microcorys F. Muell.) to decay. Aust. J. biol. Sci. 2: 45-57.

Darbyshire, B. (1967). Factors influencing the susceptibility of apple trees to Trametes (Polystictus) versicolor. Ph.D. thesis, University of Tasmania.

Darbyshire, B. Wade, G.C. and Marshall, K.C. (1969). In vitro studies of the role of nitrogen and sugars on the susceptibility of apple wood to decay by Trametes versicolor. Phytopathology 59: 98-102.

Davis, M.B. (1930). Investigations on the nutrition of fruit trees. Some effects of deficiencies of nitrogen, phosphorus, calcium and magnesium with special reference to the behaviour of certain varieties of apple trees. J. Pom. hort. Sci. 8: 316-344.

de Bruyn, J.W., Van Keulen, H.A. and Ferguson, J.H.A. (1968). Rapid method for the simultaneous determination of glucose and fructose using anthrone reagent. J. Sci. Fd. Agric. 19: 597-601.

Dimond, A.E. (1955). Pathogenesis in the wilt diseases. <u>Ann. Rev. Pl.</u> <u>Physiol.</u> <u>6</u>: 329-350.

Doepel, R.F. (1962). Wood-rotting fungi associated with dieback in apple trees. <u>J. Dep. Agric. West Aust. (ser.4)</u> <u>3</u>: 668-678.

Doepel, R.F. (1965). Apple dieback in Western Australia. <u>J. Dep. Agric.</u> <u>West Aust. (ser.4)</u> <u>6</u>: 82-88.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. <u>Analyt. Chem.</u> <u>28</u>: 350-356.

Dutton, G.G.S. and Murata, T.G. (1961). The constitution of the hemi-cellulose of apple wood (<u>Malus</u> <u>pumila</u> L. var. Golden Transparent). <u>Can. J. Chem.</u> <u>39</u>: 1995-2000.

Edgren, J.W. (1959). Analysis of non-destructive methods of detecting decay in living trees. M.S. thesis, Iowa State University of Science and Technology.

Eide, C.J. and Christensen, C.M. (1940). Wood decay in apple trees in Minnesota. <u>Phytopathology</u> <u>30</u>: 936-944.

Fidler, J.C. (1961). Physiological activity of volatile organic compounds produced by fruit. <u>Bull. int. Inst. Refrig., Annexe 1961-63</u>: 379-389.

Findlay, W.P.K. (1934). Studies in the physiology of the wood destroying fungi. I. The effect of nitrogen content upon the rate of decay of timber. <u>Ann. Bot.</u> <u>48</u>: 109-117.

Findlay, W.P.K. (1941). Effect of addition of sugar on the rate of decay of wood. <u>Ann. appl. Biol.</u> <u>28</u>: 19-22.

Fisher, E. (1934). Observations on <u>Fomes</u> <u>pomaceous</u> (Pers.) Big. & Guill. infecting plum trees. <u>Trans. Br. mycol. Soc.</u> <u>19</u>: 102-113.

Frank, A.B. (1884). Ueber die Gummibildung in Holze und deren physiologische Bedeutung. <u>Ber. deutsch. Bot. Ges.</u> <u>2</u>: 321-332.

Frey-Wyssling, A. and Bosshard, H.H. (1959). Cytology of the ray cells in sapwood and heartwood. <u>Holzforschung</u> <u>13</u>: 129-137.

Gagnon, C. (1967). Polyphenols and discoloration in the elm disease investigated by histochemical techniques. <u>Can. J. Bot.</u> <u>45</u>: 2119-2124.

Garber, E.D. (1956). A nutrition-inhibition hypothesis of pathogenicity. Am. Nat. 90: 183-194.

Gäumann, E. (1950). Principles of plant infection. (Authorised English Edition by W.B. Brierley.) Crosby Lockwood & Son, Ltd., London.

Good, H.M. and Nelson, C.D. (1951). A histological study of sugar maple decayed by Polyporus glomeratus Peck. Can. J. Bot. 29: 215-224.

Good, H.M., Murray, P.M. and Dale, H.M. (1955). Studies on heartwood formation and staining in sugar maple, Acer saccharum Marsh. Can. J. Bot. 33: 31-41.

Good, H.M., Basham, J.T. and Kadzielawa, S.D. (1968). Respiratory activity of fungal associations in zones of heart rot and stain in sugar maple. Can. J. Bot. 46: 27-36.

Goode, J.E. and Hyrycz, K.J. (1964). The response of Laxton's Superb apple trees to different soil moisture conditions. J. hort. Sci. 39: 254-276.

Greaves, H. and Levy, J.F. (1965). Comparative degradation of the sapwood of Scots pine, beech and birch by Lenzites trabea, Polystictus versicolor, Chaetomium globosum and Bacillus polymyxa. J. Inst. Wood Sci. 3: 55-63.

Grosclaude, C. (1960). Le plomb des arbres fruitiers I. État actuel de la question. Ann. Epiphyties 11: 397-417.

Grosclaude, C. (1961). Le plomb des arbres fruitiers. II. Travaux préliminaires réalisés a la Station de Pathologie végétale. Centre de Recherches agronomiques du Sud-Ouest Pont-de-la-Maye. Ann. Epiphyties 12: 75-87.

Grosclaude, C. (1962). Le plomb des arbres fruitiers. III. Le plomb tardif du pécher. Ann. Epiphyties 13: 167-177.

Grosclaude, C. (1964a). Le plomb des arbres fruitiers. IV. L'infection du pêcher par le Stereum purpureum. Résultats d'inoculations artificielles et d'assais in vitro. Ann. Epiphyties 15: 5-20.

Grosclaude, C. (1964b). Le plomb des arbres fruitiers. V. L'infection latente du pêcher par le Stereum purpureum. Ann. Epiphyties 15: 431-438.

Grosclaude, C. (1966a). La gommose des arbres fruitiers. Ann. Epiphyties 17: 129-137.

Grosclaude, C. (1966b). Le bois du pêcher, principales altérations d'origine fongique ou traumatique. Ann. Epiphyties 17: 263-283.

Grosclaude, C. (1967). Le plomb des arbres fruitiers. VI. Note sur les effets conjugués du Stereum purpureum et de l'asphyxie des racines chez le prunier GF 8-1. Ann. Epiphyties 18: 331-334.

Grosclaude, C. (1968). Considerations preliminaires à l'etude de la resistance au 'Plomb' des arbres fruitiers. Revue Zool. agric. appl. No.7-9: 73-81.

Grosjeahn, J. (1955). Periodicite annuelle de l'activite du Stereum purpureum. T. Pl. Zeikten. 62: 226-235.

Haas, G.J. and Fleischman, A.I. (1958). Use of the anthrone procedure in the carbohydrate analysis of beer. Wallerstein Lab. Commun. 21: 139-151.

Hageman, R.H. (1969). Phosphorus metabolism in plants. Hortscience 4: 311-314.

Handbook of Physics and Chemistry (1944). 28th Ed. Chemical Rubber Publishing Co., Cleveland.

Harrison, A.F. and Clare, B.G. (1970). Host reactions involved in the recovery of apricot trees from Verticillium wilt. Aust. J. biol. Sci. 23: 1027-1032.

Hart, J.H. (1963). Development of wound heartwood in Iowa hardwoods. Ph.D. thesis, Iowa State University of Science and Technology.

Hart, J.H. (1964). Production of fungistatic substances by mechanically damaged sapwood. Phytopathology 54: 895.

Hart, J.H. (1965). Formation of discolored sapwood in three species of hardwoods. Quarterly Bull. Mich. Agr. Exp. Sta. 48: 101-116.

Hart, J.H. (1968). Morphological and chemical differences between sapwood, discolored sapwood and heartwood in black locust and osage orange. Forest. Sci. 14: 344-338.

Hart, J.H. and Johnson, K.C. (1970). Production of decay-resistant sapwood in response to injury. Wood Sci. Technology 4: 267-272.

Hartley, C., Davidson, R.W. and Crandall, B.S. (1961). Wetwood, bacteria and increased pH. U.S. Forest Prod. Lab Rep. 2215. 34pp.

Hasegawa, M. and Shirato, T. (1959). Abnormal constituents of Prunus wood: Isoolivil from Prunus jamasakura wood. J. Jap. For. Soc. 41: 1-3.

Hassid, W.Z. and Neufield, E.F. (1964). Quantitative determination of starch in plant tissues. In Methods of Carbohydrate Chemistry (R.L. Whistler, Ed.). Vol. IV. Academic Press, New York.

Hepting, G.H. and Blaisdell, Dorothy J. (1936). A protective zone in red gum fire scars. Phytopathology 26: 62-67.

Hewitt, E.J. (1966). Sand and water culture methods used in the study of plant nutrition. 2nd Ed. Comm. Agric. Bur. Tech. Comm. 22.

Hibbard, A.D. and Nour, M. (1959). Leaf content of phosphorus and potassium under moisture stress. Proc. Am. Soc. hort. Sci. 73: 33-39.

Higgins, B.B. (1919). Gum formation with special reference to cankers and decays of woody plants. Ga. Exp. Sta. Bull.127: 1-59.

Hillis, W.E. (1962). Wood extractives and their significance to the pulp and paper industries. Academic Press, London.

Hillis, W.E. (1968). Chemical aspects of heartwood formation. Wood Sci. Technology 2: 241-259.

Hillis, W.E. and Inoue, T. (1968). The formation of polyphenols in trees. IV. The polyphenols formed in Pinus radiata after Sirex attack. Phytochem. 7: 13-22.

Hillis, W.E. and Swain, T. (1959). The phenolic constituents of Prunus domestica. II. The analysis of tissues of the Victoria plum tree. J. Sci. Fd. Agric. 10: 135-144.

Hirt, R.R. and Lowe, J.L. (1945). *Polyporus versicolor* on Asiatic chestnut. Phytopathology 35: 574-575.

Hossfeld, R.L., Oberg, J.C. and French, D.W. (1957). The appearance and decay resistance of discolored aspen. Forest Prod. J. 7: 378-382.

Hough, L. and Pridham, J.B. (1959). The composition of plum gums. Biochem. J. 73: 550-559.

Howard, B.H. and Banwell, M.G. (1969). Avoid holding maiden trees in cold stores containing fruit. Comm. Gr. No. 3854: 453-455.

Jacob, K.D. and Hoffman, W.M. (1954). Report on phosphoric acid in fertilizers – determination of available phosphoric acid. J. Ass. off. agric. Chem. 37: 334-359.

Jacobs, W.P. (1950). Auxin transport in the hypocotyl of *Phaseolus vulgaris*. Am. J. Bot. 37: 248-254.

Jensen, W.A. (1902). Botanical histochemistry. W.H. Freeman and Co., San Francisco.

Jorgensen, E. (1961). The formation of penosylvin and its monomethyl ether in the sapwood of *Pinus resinosa* Ait. Can. J. Bot. 39: 1765-1772.

Jorgensen, E. (1962). Observations on the formation of protection wood. For. Chron. 38: 292-294.

Jørstad, I. (1948). Lignicolous and corticolous Heterobasidiomycetae and Aphyllophoraceae on fruit trees and small fruits in Norway. Friesia 3: 352-376.

Kaufert, F.H. and Behr, E.A. (1942). Susceptibility of wood to decay. Effect of urea and other nitrogen compounds. Ind. Eng. Chem. 34: 1510-1515.

Kenworthy, A.L. (1949). Soil moisture and growth of apple trees. Proc. Am. Soc. hort. Sci. 54: 29-39.

Klepper, Betty (1968). Diurnal pattern of water potential in woody plants. Pl. Physiol. 43: 1931-1934.

Klepper, Betty and Ceccato, R.D. (1969). Determination of leaf and fruit water potential with a pressure chamber. Hort. Res. 9: 1-7.

Lafuse, H.H. (1937). Nutritional characteristics of certain wood-destroying fungi, Polyporus betulinus Fr., Fomes pinicola (Fr.) Cooke, and Polystictus versicolor Fr. Pl. Physiol. 12: 625-646.

Lamp, C.A. (1968). The effect of potassium upon the uptake and distribution of magnesium in plants. Ph.D. thesis, University of Tasmania.

Levi, M.P. and Cowling, E.B. (1968). Role of nitrogen in wood deterioration. V. Changes in decay susceptibility of oak sapwood with season of cutting. Phytopathology 58: 246-249.

Levi, M.P., Merrill, W. and Cowling, E.B. (1968). Role of nitrogen in wood deterioration. VI. Mycelial fractions and model nitrogen compounds as substrates for growth of Polyporus versicolor and other wood-destroying and wood-inhibiting fungi. Phytopathology 58: 626-634.

Lorenz, R.C. (1944). Discoloration and decay resulting from increment borings in hardwoods. J. Forest. 42: 37-43.

Lyon, C. and Garcia, C.R. (1944). Anatomical responses in the anatomy of tomato stems in relation to variations in the macronutrient cation supply. Bot. Gaz. 105: 441-456.

Lyr, H. (1967). Über den jahrezeitlichen Verlauf der Schutzkernbildung bei Pinus silvestris nach Verwundungen. Arch. Fortshw. 16: 51-57.

Magness, J.R., Regeimbal, L.O. and Degman, E.S. (1932). Accumulation of carbohydrate in apple foliage, bark, and wood as influenced by moisture supply. Proc. Am. Soc. hort. Sci. 29: 246-252.

Manion, P.D. and French, D.W. (1969). The role of glucose in stimulating germination of Fomes igniarius var. populinis basidiospores. Phytopathology 59: 293-296.

Markham, R. (1942). A steam distillation apparatus suitable for micro-Kjeldahl analysis. Biochem. J. 36: 790-791.

Martin, J.P. (1950). The use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69: 215-232.

Mason, A.C. (1958). The effect of soil moisture on the mineral composition of apple plants grown in pots. J. hort. Sci. 33: 202-211.

McNabb, H.S. Jr., Edgren, J.W. and Eslyn, W.E. (1959). Pathological and normal heartwood in hardwoods. Proc. Ninth Int. Bot. Congr. 2: 244-245.

Merrill, W. (1970). Spore germination and host penetration by heart-rotting Hymenomycetes. Ann. Rev. Phytopath. 8: 281-300.

Merrill, W. and Cowling, E.B. (1966a). Role of nitrogen in wood deterioration: Amounts and distribution of nitrogen in tree stems. Can. J. Bot. 44: 1555-1580.

Merrill, W. and Cowling, E.B. (1966b). Role of nitrogen in wood deterioration. IV. Relationship of natural variation in nitrogen content of wood to its susceptibility to decay. Phytopathology 56: 1324-1325.

Mochizuki, T. (1962). Studies on the elucidation of factors affecting the decline in tree vigour in apples as induced by fruit load. Bull. Fac. Agric. Hirosaki Univ. No.8: 40-124.

Mochizuki, T. (1963). The effects of varying moisture supply on the nutritional status of apple trees. Bull. Fac. Agric. Hirosaki Univ. No.9: 21-30.

Mochizuki, T. and Hanada, S. (1958). The phosphorus nutrition of apple trees. Part I Effects of phosphorus concentration in culture solutions. Bull. Fac. Agric. Hirosaki Univ. No.4: 1-18.

Münch, E. (1910). Über krankhafte Kernbildung. Naturw. Ztschr. Forst-u. Landwirtsch 8: 533-547, 553-569.

Nutman, F.J. (1929). Studies of wood-destroying fungi. I. Polyporus hispidis (Fries). Ann. appl. Biol. 16: 40-64.

Ohman, J.H. and Kessler, K.J. (1964). White trunk rot of hardwoods. U.S. Dept. Agric. For. Pest Leafl. 88: 7pp.

Palct, J. (1967). Über pathologische Veranderungen im Stamm von Prunus armenaciae L. und ihre Begiehung zum fruhzeitigen Marillensterben I. Untersuchungen im Bereich des Holzgewebes. Phytopath. Z. 60: 237-246.

Peace, T.R. (1962). Pathology of trees and shrubs. Oxford University Press, London.

Peterson, C.A. and Cowling, E.B. (1964). Decay resistance of extractive-free coniferous woods to white rot fungi. Phytopathology 54: 542-547.

Pickard, M.A. and Westlake, D.W.S. (1969). Metabolism of rutin by Polyporus versicolor. PRL 572. Can. J. Microbiol. 15: 869-873.

Platt, W.D., Cowling, E.B. and Hodges, C.S. (1965). Comparative resistance of coniferous root wood and stem wood to decay by isolates of Fomes annosus. Phytopathology 55: 1347-1353.

Prael, E. (1888). Vergleichende Untersuchungen über Schütz und Kernholz der Laubbaume. Jahrb. Wiss. Bot. 19: 1-81.

Priestley, C.A. (1962a). Carbohydrate resources within the perennial plant. Comm. Agric. Bur. Tech. Comm. No.27.

Priestley, C.A. (1962b). The location of carbohydrate resources within the apple tree. Proc. 16th Int. Hort. Congr., Brussels 3: 319-327.

Prillieux, E. (1875). Etude sur la formation de la gomme dans le arbres fruitiers. Ann. des Sci. Nat. Bot. Ser. 6: 176-200.

Rankin, W. (1933). Wound gums and their relation to fungi. Nat. Shade Tree Conf. Proc. 9: 111-115.

Rawlins, T.E. and Takahashi, W.N. (1952). Technics of plant histochemistry and virology. The National Press, Millbrae, California.

Reeve, R.M. (1959a). A specific hydroxylmaine-ferric chloride reaction for histochemical localisation of pectin. Stain Technol. 34: 209-211.

Reeve, R.M. (1959b). Histological and histochemical changes in developing and ripening peaches. III. Catechol tannin content per cell. Am. J. Bot. 46: 645-650.

Reid, M.S. and Bieleski, R.L. (1970). Response of Spirodela oligorrhiza to phosphorus deficiency. Pl. Physiol. 46: 609-613.

Rhoads, A. (1917). The black zones formed by wood-destroying fungi. N.Y. State Coll. Forestry Syracuse, Tech. Pub. No.8: 1-61.

Rosik, J. Kubala, J., Stanová, M. and Lačok, P. (1968). Structural properties of polysaccharides from apricot-tree gum in dependence on infection of the tree by pathogens or application of 2-methyl-4-chlorophenoxyacetic acid. Collection Czechoslov. Chem. Commun. 33: 1943-1945.

Sachs, I.B., Ward, J.C. and Bulgrin, E.H. (1966). Heartwood stain in red oak. Holz. Roh. Werkstoff 24: 489-497.

Scheffer, T.C. and Cowling, E.B. (1966). Natural resistance of wood to microbial deterioration. Ann. Rev. Phytopath. 4: 147-170.

Scheffer, T.C. and Livingston, B.E. (1937). Relation of oxygen pressure and temperature to growth and carbon-dioxide production in the fungus Polystictus versicolor. Am. J. Bot. 24: 109-119.

Schmitz, H. and Kaufert, F. (1936). The effect of certain nitrogenous compounds on the rate of decay of wood. Am. J. Bot. 23: 635-638.

Schmitz, H. and Kaufert, F. (1938). Studies in wood decay. VIII. The effect of the addition of dextrose and dentrose and asparagine on the rate of decay of Norway pine sapwood by Lenzites trabea and Lentinus lepideus. Am. J. Bot. 25: 443-448.

Shain, L. (1967). Resistance of sapwood in stems of loblolly pine to infection by Fomes annosus. Phytopathology 57: 1034-1045.

Shain, L. (1971). The response of sapwood of Norway spruce to infection by Fomes annosus. Phytopathology 61: 301-307.

Shigo, A.L. (1965). The pattern of decays and discolorations in northern hardwoods. Phytopathology 55: 648-652.

Shigo, A.L. (1967a). The early stages of discoloration and decay in living hardwoods in north-eastern United States: a consideration of wound-initiated discoloration and heartwood. 14th Int. Union Forest. Res. Organ. Congr., Munich, Proc. 9, Sect. 4: 117-133.

Shigo, A.L. (1967b). Successions of organisms in the discoloration and decay of wood. Int. Rev. Forest. Res. 2: 237-299.

Shigo, A.L. and Sharon, E.M. (1968). Discoloration and decay in hardwoods following inoculations with Hymenomycetes. Phytopathology 58: 1493-1498.

Shigo, A.L. and Sharon, E.M. (1970). Mapping columns of discolored and decayed tissues in sugar maple. *Acer saccharum*. Phytopathology 60: 232-237.

Singh, J.N. and Singh, D.P. (1968). Effect of phosphorus deficiency on carbohydrate metabolism of *Mentha arvensis*. Physiologia Pl. 21: 1341-1347.

Smith, E.C. (1930). *Trametes hispida* a destructive parasite in apple orchards. Mycologia 22: 221-223.

Smith, F. and Montgomery, R. (1959). The chemistry of plant gums and mucilages. Reinhold Publishing Corp., New York.

Somers, T.C. and Harrison, A.F. (1967). Wood tannins - isolation and significance in host resistance to *Verticillium* wilt disease. Aust. J. biol. Sci. 20: 475-479.

Sommer, Anna L. (1936). The relationship of phosphate concentration of solution cultures to the type and size of root systems and the time of maturity of certain plants. J. agric. Res. 52: 133-148.

Splittstoesser, W.E. (1969). Arginine metabolism by pumpkin seedlings. Separation of plant extracts by ion exchange resins. Pl. Cell Physiol. 10: 87-94.

Steel, R.G.D. and Torrie, J.H. (1960). Principles and procedures of statistics. M Graw-Hill Book Company, Inc., New York.

Sterling, C. (1970). Crystal structure of ruthenium red and stereochemistry of its pectin stain. Am. J. Bot. 57: 172-175.

Stewart, C.M. (1966). Excretion and heartwood formation in living trees. Science 153: 1068-1074.

Stewart, C.M. (1967). Moisture content of living trees. Nature 214: 138-140.

Sucoff, E., Ratsch, H. and Hook, D.D. (1967). Early development of wound-initiated discoloration in *Populus tremiloides* Michx. Can. J. Bot. 45: 649-656.

Swain, T. and Hillis, W.E. (1959). The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. J. Sci. Fd. Agric. 10: 63-68.

Swarbrick, T. (1926). The healing of wounds in woody stems.
J. Pom. hort. Sci. 5: 98-114.

Talboys, P.W. (1968). Water deficits in vascular disease. In Water
deficits and plant growth. Vol.II. (T. Kozlowski, Ed.)
Academic Press, New York.

Tattar, T.A., Shortle, W.C. and Rich, A.E. (1971). Sequence of micro-
organisms and changes in constituents associated with
discoloration and decay of sugar maples infected with
Fomes connatus. Phytopathology 61: 556-558.

Temme, F. (1885). Über Schutz-und Kernholz, seine Bildung und Physiologische
Bedeutung. Landwirtsch. Jahrb. 14: 465-485.

Thomas, P.H. and Raphael, T.D. (1933). Re-working full-bearing apple
trees. Tasm. J. Agric. 4: 147-156.

Truog, E., Goates, R.J., Gerloff, G.C. and Berger, K.C. (1947).
Magnesium-phosphorus relationships in plant nutrition.
Soil Sci. 63: 19-25.

van der Weij, H.G. (1932). Der Mechanismus des Wuchsstoff-transportes.
Rec. Trav. bot. neerl. 29: 379-496.

von Schrenk, H. and Spaulding, P. (1909). Diseases of deciduous forest
trees. U.S. Dept. Agr. Bur. Pl. Ind. Bull. 149: 85pp.

Wade, G.C. (1968). The influence of mineral nutrition on the suscepti-
bility of apple trees to infection by Trametes (Polystictus)
versicolor. Aust. J. exp. Agric. Anim. Husb. 8: 436-439.

Wagener, W.W. and Davidson, R.W. (1954). Heart rots in living trees.
Bot. Rev. 20: 61-134.

Wallace, T. (1951). The diagnosis of mineral deficiencies in plants by
visual symptoms. 2nd Ed. His Majesty's Stationary Office,
London.

Ward, J.R. (1957). Winter control of orchard diseases. Tasm. J. Agric.
28: 127-134.

Wardell, J.F. and Hart, J.H. (1970a). Early responses of sapwood of
Quercus bicolor to mechanical injury. Can. J. Bot. 48:
683-686.

Wardell, J.F. and Hart, J.H. (1970b). Determination of the distribution of inorganic elements in the wood of white oak with the microprobe. Abstracts First North American Forest Biology Workshop, Michigan State University, East Lansing.

Weir, J.R. (1923). The genus *Polystictus* and the decay of living trees. *Phytopathology* 13: 184-186.

West, D.W., Thompson, W.K. and Black, J.D.F. (1970). Polar and lateral transport of water in an apple tree. *Aust. J. biol. Sci.* 23: 231-234.

White, J.W. and Mastalerz, J.W. (1966). Soil moisture as related to container capacity. *Proc. Am. Soc. hort. Sci.* 89: 758-765.

Wiersum, L.K. (1966). Calcium content of fruits and storage tissues in relation to the mode of water supply. *Acta bot. neerl.* 15: 406-418.

Willison, R.S. (1932). Wound gum in peaches and grapes. Its relation to the invasion of fungus wound-parasites. *Scient. Agric.* 12: 402-419, 484-505.

Winston, P.W. and Bates, D.H. (1960). Saturated solutions for the control of humidity in biological research. *Ecology* 41: 232-237.

Wormald, H. (1955). Diseases of fruits and hops. 3rd Ed. Crosby Lockwood and Son Ltd., London.

Yokomizo, H., Yamazaki, T., Mori, H. and Fukuda, H. (1964). The accumulation of minerals and carbohydrates of apple trees grown in sand culture and their translocation in the following spring. *Bull. Hort. Res. Sta. Japan Series C (Morioka)* No.2: 55-67.

Ziegler, H. (1964). Storage, mobilisation and distribution of reserve material in trees. *In* The formation of wood in forest trees. (M. Zimmerman, Ed.) Academic Press, New York.

Ziegler, H. (1967). Biologische Aspekte der Kernholzbildung. *14th Int. Union Forest. Res. Organ. Congr.*, Munich, Proc. 9, Sect. 41: 93-116.

Zitko, V., Rosik, J., Brutenicova, M. and Kubala, J. (1965). *Collection Czecholsov. Chem. Commun.* 30: 3501.

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APPENDICES

APPENDICES I(a), (b), (c).

Data on which Table 12 is based.

Appendix I(a)

Depth (mm) below wound surface at which gum formation commenced in wounds 30 and 120 days old in three seasons.

Rep.	July (J)		December (D)		April (A)	
	30 day	120 day	30 day	120 day	30 day	120 day
1	1.8	1.7	4.6	3.8	1.5	1.7
2	1.8	1.5	2.3	5.9	2.0	1.6
3	2.1	2.0	4.8	4.6	1.5	1.5
4	2.1	1.3	3.4	5.4	1.7	2.2
5	2.2	1.4	3.2	3.8	1.5	2.0
6	1.4	2.3	6.4	5.5	3.4	1.5
7	1.5	2.6	5.2	4.5	1.1	1.5
8	2.3	2.8	5.4	4.0	1.6	0.8
9	1.1	2.7	5.7	3.8	2.1	2.4
10	1.2	2.3	6.0	2.7	0.8	1.7
11	1.2	2.2	4.2	2.6	2.4	2.7
12	1.7	1.8	5.1	3.6	1.5	3.5
13	2.4	1.5	5.7	4.1	1.6	1.3
14	1.4	2.5	3.4	3.4	2.7	1.3
15	1.3	1.5	3.1	2.9	2.4	1.4
16	2.1	3.3	4.1	5.1	2.0	1.3
17	1.2	1.4	4.7	2.9	2.0	1.8
18	1.5	2.2	3.7	4.5	2.4	1.7

Appendix I(a) cont.

Analysis of variance

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Season (S)	2	141.47	70.74	118.89***
Age of wound (A)	1	0.11	0.11	0.185 n.s.
S x A	2	3.02	1.51	2.54 n.s.
Trees (T)	17	9.13 ⎫		
A x T	17	5.94 ⎬ 60.65†	0.595	
S x T	34	17.31 ⎬		
S x A x T	34	28.27 ⎭		
Total	107	205.25		

† Bartlett's test for homogeneity of variances comprising error term was not significant.

Appendix I(b)

Length of discoloration (mm) beneath wounds 120 days old, in three seasons.

Rep.	July (J)	December (D)	April (A)
1	5.5	5.0	3.0
2	4.0	11.0	3.5
3	5.5	11.0	2.5
4	4.5	9.0	4.5
5	4.5	6.5	4.0
6	5.3	8.5	3.5
7	5.5	10.0	3.0
8	6.0	9.5	5.0
9	5.0	6.0	3.5
10	4.0	9.0	4.5
11	5.0	9.5	4.5
12	4.0	6.5	5.0
13	3.0	9.5	2.5
14	4.5	13.0	2.0
15	3.5	8.5	3.0
16	4.0	9.0	3.0
17	4.5	10.5	3.5
18	4.0	10.5	4.0

Analysis of variance

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Season (S)	2	302.80	151.40	67.59***
Trees (T)	17	23.33	1.37	<1 n.s.
Error (S x T)	34	68.81	2.24	
Total	53	394.94		

Appendix I(c)

Length of gum formation (mm) in wounds 120 days old, in three seasons.

Rep.	July (J)	December (D)	April (A)
1	5.5	8.5	3.4
2	5.8	10.0	1.9
3	7.6	11.0	4.1
4	7.7	10.9	3.4
5	3.5	11.0	3.0
6	3.6	9.0	2.5
7	5.3	8.0	2.9
8	4.2	6.0	3.4
9	6.0	6.5	3.2
10	8.3	9.0	3.2
11	4.3	12.3	3.0
12	6.0	8.4	2.5
13	2.3	14.2	2.7
14	7.5	10.7	2.5
15	4.1	5.0	2.0
16	3.9	8.1	4.1
17	3.0	10.0	3.0
18	3.5	13.2	8.2

Analysis of variance

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Season (S)	2	373.55	186.78	52.32***
Trees (T)	17	65.54	3.86	1.08 n.s.
Error (S x T)	34	121.38	3.57	
Total	53	560.47		

APPENDICES I(d), (e)

Data on which Table 14 is based.

APPENDIX I(d)

Length of penetration (mm.) by T. versicolor in inoculations in 5-year-old STP limbs. Wounds were made in three seasons and inoculated at 0, 15 and 30 days after wounding in each season.

Rep.	July (J) 0	15	30	December (D) 0	15	30	April (A) 0	15	30
1	4.3	4.0	2.5	8.5	3.8	2.0	6.6	1.7	1.4
2	5.2	2.0	4.8	18.7	4.8	2.4	6.2	3.0	1.8
3	6.1	2.8	6.1	9.0	3.2	2.7	1.8	0.7	2.7
4	11.7	2.3	7.6	10.1	4.8	3.8	3.7	0.9	1.4
5	9.6	5.5	3.6	7.5	6.7	3.4	2.0	0.7	1.3
6	8.5	3.7	2.5	10.3	4.7	1.3	3.2	2.4	1.4
7	5.0	1.6	5.3	6.7	6.0	4.3	3.2	1.6	1.9
8	5.6	3.6	1.2	12.9	2.8	1.7	3.4	0.9	1.4
9	4.0	2.4	4.6	6.2	5.4	4.0	7.2	1.7	1.4
10	7.5	3.4	2.7	8.4	3.4	3.2	3.3	2.5	3.2
11	3.9	4.9	1.2	5.3	3.2	2.2	3.5	2.5	2.5
12	4.3	3.2	4.0	10.7	4.2	4.3	2.8	1.9	1.7
13	8.6	2.2	1.1	6.2	4.1	2.2	2.9	1.2	2.4
14	3.2	1.6	1.6	7.7	2.7	3.2	4.4	1.5	1.0
15	3.5	3.0	3.8	13.1	6.6	2.8	2.1	1.3	1.5
16	8.6	3.3	2.1	5.2	8.7	2.4	2.4	1.2	1.3
17	8.5	4.5	2.5	6.6	5.7	2.1	1.4	3.2	2.9
18	5.8	1.4	1.1	3.8	4.0	2.5	4.4	1.6	2.8

Analysis of Variance

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Season (S)	2	250.19	125.10	36.7***
Age of wound (A)	2	400.79	200.40	58.8***
S x A	4	86.55	21.64	6.4***
Trees (T)	17	60.30)		
A x T	34	103.17)	3.41	
S x T	34	119.25) 521.39[†]		
S x A x T	68	238.67)		
Total	161	1258.92		

† Bartlett's test for homogeneity of variances comprising error was not significant.

APPENDIX I(e)

Depth (mm.) below point of inoculation at which gum formation commenced in inoculated wounds in 5-year-old STP limbs. Wounds were made in three seasons and inoculated at 0, 15 and 30 days after wounding in each season.

Rep.	July (J)			December (D)			April (A)		
	0	15	30	0	15	30	0	15	30
1	6.8	4.8	2.5	11.6	5.9	4.0	8.2	4.0	1.4
2	9.1	2.0	4.8	28.2	8.0	5.3	8.2	6.0	1.8
3	6.1	5.0	2.5	13.7	6.7	2.7	3.6	2.1	2.7
4	14.4	2.3	6.1	14.8	8.2	4.7	6.4	3.0	1.4
5	12.8	5.5	7.6	9.5	10.5	4.8	3.9	3.6	1.3
6	10.5	5.2	3.6	12.7	9.9	2.4	7.8	5.9	1.4
7	5.0	3.1	2.5	10.5	7.8	6.0	4.6	3.7	1.9
8	5.6	3.6	5.3	15.0	5.3	3.6	7.0	2.2	1.4
9	7.2	2.4	1.2	13.2	10.4	5.0	9.6	5.5	1.4
10	8.8	6.0	4.6	12.6	5.8	4.9	5.7	4.9	3.2
11	6.6	6.0	2.7	7.8	4.6	4.4	6.8	4.5	2.5
12	6.9	5.5	1.2	14.9	12.2	5.3	6.9	4.7	1.7
13	13.6	3.5	4.0	10.6	4.1	2.8	5.2	2.6	2.4
14	8.0	2.9	1.1	11.0	5.9	2.2	5.7	4.1	1.0
15	6.0	4.1	1.6	16.6	9.5	4.9	4.9	3.1	1.5
16	8.6	5.8	3.8	8.5	16.7	4.2	5.5	3.2	1.3
17	8.5	5.2	2.1	8.6	6.9	4.1	3.8	5.5	2.9
18	8.5	2.9	1.1	6.4	7.5	7.2	6.6	5.1	2.8

Analysis of Variance

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Season (S)	2	532.98	266.49	46.27***
Age of wound (A)	2	950.54	475.27	82.51***
S X A	4	93.39	23.35	4.05**
Trees (T)	17	114.27)		
A x T	34	211.95)	881.56† 5.76	
S x T	34	232.60)		
S x A x T	68	322.74)		
Total	161	2458.27		

† Bartlett's test for homogeneity of variances comprising error term was not significant.

APPENDIX I(f)

Data on which Table 15 is based. Length of gum formed beneath inoculations in 5-year-old STP limbs. Wounds were made in three seasons and inoculated at 0, 15 and 30 days after wounding in each season.

Rep.	July (J)			December (D)			April (A)		
	0	15	30	0	15	30	0	15	30
1	7.2	6.3	2.5	18.0	11.0	20.2	4.5	2.9	5.7
2	10.0	5.2	3.9	19.0	19.0	10.5	9.5	4.7	2.7
3	7.0	4.4	6.5	16.4	15.9	13.0	3.3	2.8	6.8
4	7.1	3.1	4.8	15.8	10.4	9.8	4.6	3.5	4.1
5	7.8	4.6	10.2	12.3	8.7	20.0	5.6	4.2	4.5
6	7.5	2.7	4.7	13.2	14.8	9.2	9.7	4.0	3.9
7	5.5	3.1	3.8	15.8	9.3	17.8	5.8	4.3	6.4
8	8.9	4.8	3.6	19.8	9.7	11.7	5.0	4.7	4.9
9	9.0	3.8	4.7	10.7	6.7	16.4	6.1	4.0	3.2
10	4.0	3.3	6.0	15.6	8.4	24.2	3.7	5.2	4.9
11	6.8	6.6	6.9	4.4	6.0	6.7	7.6	6.1	7.2
12	7.8	6.0	2.3	24.0	14.0	12.9	3.9	3.1	5.0
13	8.0	3.7	4.8	6.8	4.3	12.3	3.8	3.6	3.6
14	7.4	2.3	4.7	16.0	10.2	18.8	6.0	3.4	6.5
15	7.3	2.8	5.8	14.1	21.8	8.2	3.7	5.0	4.6
16	9.2	5.0	9.3	6.0	12.8	12.7	3.7	4.9	5.0
17	12.0	6.3	4.1	7.8	8.6	12.2	2.9	3.8	5.5
18	3.6	2.5	3.2	12.5	18.5	5.5	4.2	4.9	4.9

Analysis of Variance

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Season (S)	2	2188.82	1094.41	106.36***
Age of wound(A)	2	125.18	62.59	6.08**
S x A	4	38.27	9.57	0.93 n.s.
Trees (T)	17	140.96)		
A x T	34	385.41)		
S x T	34	462.58) 1573.80†	10.29	
S x A x T	68	584.85)		
Total	161	3926.07		

† Bartlett's test for homogeneity of variance comprising error term was not significant.

APPENDIX 2(a)

Analysis Hobart Water Supply.

Reaction (pH)   6.7-7.2
Color (Hazen units)   Nil

	Milligrams per litre
Total dissolved solids ($105^{\circ}C$)	25-54
Total dissolved solids ($500^{\circ}C$)	14-38
Total hardness (calc. as $CaCO_3$)	9-30
Bicarbonate (HCO_3^-)	6-26
Sodium (Na)	3-6
Potassium (K)	0.2-0.8
Calcium (Ca)	2.5-7.5
Magnesium (Mg)	0.6-7.9
Phosphorus (P)	0.1-0.2
Iron and aluminium (mainly Al)	0.2-0.3
Chloride (Cl^-)	4-18
Sulphate (SO_4^{2-})	0.9-2
Silica (SiO_2)	2-8
Fluoride (F^-)	nil-1.0

APPENDIX 2(b)

Data on which Table 17 is based. Total length of shoot growth (cm.) and weight of prunings (g.) per inoculated tree for two seasons in the N x W experiment.

1968-69 season

Rep.	N_0W_0 Length	Wt.	N_0W_1 Length	Wt.	N_1W_0 Length	Wt.	N_1W_1 Length	Wt.	N_2W_0 Length	Wt.	N_2W_1 Length	Wt.
1	337	35,6	387	72.7	819	151.8	852	201.6	672	157.5	690	191.6
2	354	55.4	418	81.8	377	97.5	789	176.0	318	102.0	648	156.5
3	263	34.1	222	39.9	424	105.6	661	156.0	491	134.2	545	143.6
4	176	24.7	488	65.7	707	137.4	853	167.5	658	137.4	755	175.3
5	133	16.6	367	54.7	428	99.3	751	132.8	629	132.8	647	183.5
6	338	46.6	425	67.5	685	135.8	1050	152.5	566	135.8	589	203.2
7	314	55.1	624	64.1	674	146.2	930	159.3	765	159.3	1000	135.4
8	429	53.4	667	83.1	675	113.6	800	108.8	393	108.8	654	167.5
9	407	58.8	442	75.2	729	117.8	788	.95.7	446	95.7	760	170.7
Total	2751	380.3	4040	604.7	5518	1105.0	7474	1350.2	4938	1163.5	6288	1527.3

1969-70 season

Rep.	N_0W_0		N_0W_1		N_1W_0		N_1W_1		N_2W_0		N_2W_1	
	Length	Wt.	Length	Wt.	Length	Wt.	Length	Wt.	Length	Wt.	Length	Wt.
1	334	30	780	80	636	75	1827	220	904	90	1730	285
2	350	25	761	110	604	85	1635	285	800	105	1806	290
3	261	10	496	35	632	60	1366	180	728	90	1609	230
4	71	5	436	40	819	75	1474	215	1096	115	1725	225
5	97	5	473	32	587	70	1448	260	709	90	1420	240
6	215	10	487	50	1064	110	1591	180	595	115	1960	265
7	265	20	600	40	988	80	1529	205	1077	120	1756	250
8	453	10	521	40	809	70	1295	145	564	65	1279	185
9	221	15	532	40	848	75	1547	185	727	75	1287	230
Total	2267	130	5086	467	6987	700	13712	1875	7200	865	14572	2200

APPENDIX 2(c)

Data on which Table 18 is based. Nitrogen and phosphorus levels in the bark and wood of 3-year-old inoculated limbs at inoculation in the N x W experiment.

Nitrogen content of wood (% dry matter)

Rep.	N_0W_0	N_0W_1	N_1W_0	N_1W_1	N_2W_0	N_2W_1
1	0.308	0.318	0.361	0.371	0.441	0.444
2	0.261	0.305	0.335	0.301	0.385	0.315
3	0.169	0.195	0.361	0.396	0.361	0.431
4	0.225	0.182	0.338	0.305	0.388	0.484
5	0.235	0.285	0.358	0.351	0.335	0.332
6	0.348	0.252	0.302	0.331	0.431	0.404
7	0.212	0.248	0.367	0.272	0.493	0.401
8	0.209	0.278	0.407	0.278	0.500	0.341
9	0.252	0.232	0.308	0.325	0.407	0.354
Total	2.219	2,295	3.137	2.930	3.741	3.506

Phosphorus content of wood (ppm dry matter)

Rep.	N_0W_0	N_0W_1	N_1W_0	N_1W_1	N_2W_0	N_2W_1
1	605	543	575	560	744	650
2	642	515	578	605	668	565
3	385	505	625	685	638	653
4	378	390	605	763	665	883
5	515	383	723	660	545	550
6	510	368	585	550	763	568
7	500	348	583	495	918	563
8	493	415	758	518	913	480
9	485	595	485	578	790	608
Total	4513	4062	5517	5414	6644	5520

APPENDIX 2(c) cont.

Nitrogen content of bark (% dry matter)

Rep.	N_0W_0	N_0W_1	N_1W_0	N_1W_1	N_2W_0	N_2W_1
1	1.18	1.56	1.44	1.38	1.51	1.76
2	1.03	1.44	1.35	1.27	1.54	1.55
3	0.90	0.95	1.42	1.46	1.46	1.79
4	0.84	0.94	1.40	1.34	1.70	1.87
5	0.89	1.03	1.31	1.26	1.52	1.50
6	0.98	1.19	1.30	1.35	1.91	1.84
7	0.98	1.02	1.52	1.30	1.83	1.62
8	1.05	1.01	1.44	1.40	1.74	1.39
9	0.94	0.97	1.33	1.39	1.82	1.40
Total	8.79	10.11	12.51	12.15	15.03	14.80

Phosphorus content of bark (ppm dry matter)

Rep.	N_0W_0	N_0W_1	N_1W_0	N_1W_1	N_2W_0	N_2W_1
1	2030	2085	2190	2215	2875	2475
2	2085	2015	2135	2145	2680	1650
3	1455	1985	2670	2670	2630	2145
4	1380	1840	2740	2670	2680	2515
5	1760	1290	2610	2180	2490	2375
6	1930	1503	1985	2360	2520	2835
7	1910	1160	2040	1990	3400	2395
8	1530	1125	2855	2230	3160	2115
9	1560	1290	2260	2363	2900	2650
Total	15640	14293	21485	20823	25335	21155

APPENDIX 2(d)

Data on which Table 20 is based. Levels of soluble sugars, starch and hemicelluloses in the wood of 3-year-old inoculated limbs at inoculation in the N x W experiment.

Soluble sugars (mg./g, dry matter)

Rep.	N_0W_0	N_0W_1	N_1W_0	N_1W_1	N_2W_0	N_2W_1
1	13.15	16.73	17.11	16.92	19.73	17.44
2	16.11	15.40	18.65	17.97	17.97	17.18
3	14.31	14.79	17.25	16.08	16.21	15.79
4	14.13	13.67	16.73	15.40	15.77	16.08
5	16.02	15.44	17.84	18.37	17.31	15.83
6	15.31	16.15	19.71	16.73	17.25	18.04
7	16.27	14.79	17.84	18.72	17.25	16.27
8	14.97	15.64	16.73	16.08	17.70	16.79
9	13.05	15.31	19.00	18.37	16.95	15.15
Total	133.32	137.92	160.86	155.64	157.13	148.57

Starch content (mg./g. dry matter)

Rep.	N_0W_0	N_0W_1	N_1W_0	N_1W_1	N_2W_0	N_2W_1
1	26.47	36.47	32.22	31.02	25.18	36.70
2	36.67	27.44	22.09	40.80	21.11	31.70
3	31.61	20.85	29.39	45.86	24.22	33.28
4	14.96	26.69	18.94	26.28	24.14	30.88
5	36.67	20.32	34.83	32.90	25.37	32.10
6	30.09	24.30	35.13	35.13	30.30	36.96
7	23.68	39.99	18.94	47.79	20.24	47.80
8	25.50	29.18	36.77	23.01	27.79	39.67
9	17.63	35.87	40.80	37.66	24.99	32.80
Total	243.28	261.11	269.11	320.45	223.34	321.99

APPENDIX 2(d) cont.

Hemicelluloses (mg./g. dry matter)

Rep.	N_0W_0	N_0W_1	N_1W_0	N_1W_1	N_2W_0	N_2W_1
1	264.88	282.74	282.74	277.57	277.37	277.57
2	252.50	275.06	272.40	272.40	272.40	257.36
3	250.00	280.07	272.40	280.10	259.87	275.06
4	269.89	275.10	294.74	267.38	272.40	277.57
5	295.74	285.24	250.00	282.74	280.07	282.74
6	272.40	293.07	245.14	252.50	277.57	257.36
7	267.38	272.40	290.57	259.87	293.07	252.50
8	272.40	277.57	262.37	285.24	259.87	232.92
9	257.36	282.74	262.37	252.50	269.89	259.87
Total	2402.55	2525.99	2432.73	2430.30	2462.71	2372.95

APPENDIX 2(e)

Data on which Table 21 is based. Levels of N, P, K, Ca and Mg in leaves of inoculated trees in the N x W experiment in January 1970.

Treatment	Rep.	N	K	Ca	P	Mg
		per cent dry matter			ppm dry matter	
N_0W_0	1	1.55	1.90	0.36	1270	1880
	2	1.41	1.48	0.29	1450	1960
	3	1.13	1.66	0.33	1550	1820
	4	1.08	1.90	0.33	2384	1800
	5	1.23	1.62	0.24	1350	1340
	6	1.05	1.74	0.20	1860	1680
	7	1.40	1.92	0.26	1050	1820
	8	1.22	1.74	0.30	1420	1560
	9	1.34	1.76	0.33	2250	1780
	Total	11.41	15.72	2.64	14584	15640
N_0W_1	1	1.57	2.44	0.43	1860	1420
	2	2.18	2.68	0.51	1920	1960
	3	1.21	2.36	0.45	2360	2200
	4	1.21	2.54	0.32	2940	1660
	5	1.27	2.36	0.41	1830	1840
	6	1.74	2.58	0.37	1710	1680
	7	1.09	1.96	0.40	1680	1780
	8	1.39	2.28	0.33	1460	1960
	9	1.33	2.94	0.32	3290	1260
	Total	12.99	22.14	3.54	19050	15760
N_1W_0	1	2.34	2.06	0.43	1460	2280
	2	2.24	1.56	0.56	1340	2380
	3	1.80	1.78	0.48	1770	2260
	4	2.45	1.88	0.41	1380	1900
	5	2.21	1.82	0.45	1820	2440
	6	2.13	2.22	0.46	1400	2520
	7	2.32	1.44	0.37	1440	2000
	8	2.17	1.80	0.45	1380	2260
	9	2.20	1.96	0.43	1420	2260
	Total	19.86	16.46	4.04	13410	20300

APPENDIX 2(e) cont.

Treatment	Rep.	N	K	Ca	P	Mg
		per cent dry matter			ppm dry matter	
N_1W_1	1	2.14	2.08	0.68	1740	2680
	2	2.30	2.16	0.55	1920	2300
	3	2.18	1.90	0.48	1500	2180
	4	2.04	2.06	0.69	2290	2700
	5	2.25	2.00	0.63	1500	2600
	6	2.30	2.30	0.48	1400	2180
	7	1.97	2.20	0.57	1730	2800
	8	2.07	2.14	0.45	1480	1920
	9	2.13	2.08	0.70	1500	2840
	Total	19.38	18.92	5.23	15060	22200
N_2W_0	1	2.53	1.64	0.57	1550	2320
	2	2.63	1.76	0.49	1550	2400
	3	2.86	1.78	0.49	1540	2720
	4	2.78	1.94	0.57	1550	2540
	5	2.74	1.98	0.36	1480	2040
	6	2.54	1.78	0.48	1400	2380
	7	2.65	1.54	0.46	1650	2020
	8	2.90	1.96	0.41	1410	2320
	9	2.44	1.68	0.51	1480	2340
	Total	24.07	16.06	4.34	13610	21080
N_2W_1	1	2.62	1.86	0.62	1380	2500
	2	2.65	2.16	0.63	1680	2700
	3	2.58	1.92	0.60	1600	2460
	4	2.68	1.86	0.48	1740	2720
	5	2.66	2.06	0.52	1580	2520
	6	2.62	1.68	0.59	1540	2220
	7	2.58	1.76	0.60	1620	2780
	8	2.77	2.04	0.54	1720	2340
	9	2.59	1.86	0.58	1610	2240
	Total	23.75	17.20	5.16	14470	22480

APPENDIX 2(f)

Analysis of variance of the results on which Table 23 is based.

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Replications	8	11.34	1.42	1.14 n.s.
Treatments (T)	5	24.45	4.89	3.94**
Nitrogen (N)	2	22.95	11.47	9.24***
Water (W)	1	0.41	0.41	<1 n.s.
N x W	2	1.09	0.55	<1 n.s.
Error	40	49.64	1.24	
Total	53	85.43		
Var. between pos. of meas. (P) within treatment	36	37.11	1.03	4.91***
P	6	28.10	4.68	22.29***
P x T†	30	9.01	0.30	1.45***
Error	288	59.28	0.21	
Total	377	181.82		

† The highly significant variation between positions of measurement of hyphal penetration was expected because of the V-shaped infection front in infected limbs. As this source accounted for most of the variation considered in the variation between positions of measurement within treatment term, and because of the large number of error degrees of freedom, the position of measurement x treatment interaction was ignored.

APPENDIX 2(g)

Data on which Table 24 is based. Total length of shoot growth (cm.)
and weight of prunings (g.) per inoculated tree for three seasons in
the P experiment.

Rep.	P_0 Length	P_0 Wt.	P_1 Length	P_1 Wt.	P_2 Length	P_2 Wt.
1	656	205	905	354	559	239
2	431	131	767	314	756	295
3	504	157	507	264	792	387
4	696	243	848	313	748	296
5	648	255	1206	390	691	299
6	524	159	694	315	813	346
7	511	145	756	327	721	291
8	472	153	670	287	1028	377
9	480	163	724	292	932	366
10	534	183	780	365	867	404
11	750	283	616	259	848	387
12	685	260	650	239	634	321
Total	6871	2337	9123	3719	9399	4008
1	1116	125	1296	205	1412	215
2	646	50	1462	190	1653	225
3	626	60	1520	255	1938	240
4	661	75	1966	270	1576	225
5	806	105	1662	250	1502	215
6	644	80	1894	240	1793	255
7	716	70	1606	260	1399	220
8	882	100	1360	190	1906	275
9	871	80	1606	230	2161	310
10	982	100	1678	230	1960	280
11	912	100	1388	200	2176	270
12	975	160	1204	190	1648	205
Total	9837	1105	18642	2710	21124	2935

APPENDIX 2(g) cont.

Rep.	P_0		P_1		P_2	
	Length	Wt.	Length	Wt.	Length	Wt.
1	889	85	1993	200	1457	185
2	259	20	1031	125	1222	185
3	449	37	1910	240	1356	160
4	236	19	2022	220	1518	200
5	367	30	1293	130	1947	195
6	654	70	1571	165	1367	165
7	687	52	1430	180	1571	180
8	651	60	1329	140	1415	155
9	794	95	1244	180	1894	215
10	1042	85	1225	205	1411	205
11	542	50	1358	140	1400	170
12	908	90	1498	165	1707	155
Total	7478	693	17904	2090	18265	2170

APPENDIX 2(h)

Data on which Table 25 is based. Levels of N, K, Ca, P and Mg in leaves of inoculated trees in the P experiment in January 1970.

Treatment	Rep.	N	K	Ca	P	Mg
		per cent dry matter			ppm dry matter	
P_0	1	1.97	2.36	0.69	880	2000
	2	1.72	2.04	0.53	740	2500
	3	1.74	2.10	0.44	750	2080
	4	1.62	1.90	0.61	720	2580
	5	1.78	1.96	0.59	800	2580
	6	1.67	2.18	0.55	750	1900
	7	1.76	2.10	0.53	580	2180
	8	1.85	2.22	0.58	880	2060
	9	1.86	2.20	0.63	700	2080
	10	1.90	2.34	0.64	750	2020
	11	1.86	1.94	0.49	720	2080
	12	2.09	2.16	0.90	1150	2600
	Total	21.82	25.50	7.18	9420	26580
P_1	1	1.64	2.08	0.96	1270	2900
	2	1.68	1.68	0.76	1200	2960
	3	2.07	1.64	0.78	1370	2760
	4	1.93	2.22	0.85	1420	2600
	5	1.93	1.86	0.92	1550	3060
	6	1.97	1.92	0.95	1400	3000
	7	1.93	2.00	0.83	1160	2440
	8	1.92	1.84	0.69	1350	2180
	9	2.00	2.12	0.90	1320	3000
	10	1.71	2.14	0.71	1350	2660
	11	1.93	1.92	0.81	1500	2440
	12	2.23	2.00	1.00	1350	2480
	Total	22.94	23.42	10.16	16240	32480

APPENDIX 2(h) cont.

Treatment	Rep.	N	K	Ca	P	Mg
		per cent dry matter			ppm dry matter	
P_2	1	1.93	1.96	0.81	1830	2740
	2	2.14	2.36	0.96	1860	2720
	3	2.02	1.88	0.63	1630	2320
	4	1.95	1.90	0.92	1810	3080
	5	1.95	1.88	0.74	1770	2860
	6	1.90	2.02	0.86	2050	2960
	7	2.20	2.00	0.90	1640	2740
	8	2.10	2.18	1.04	1960	2720
	9	2.02	1.88	0.62	1740	2220
	10	2.20	1.70	0.69	1500	1940
	11	2.23	2.06	0.74	1888	2600
	12	2.03	1.86	0.76	2570	2540
	Total	24.67	23.68	9.67	22240	31440

APPENDIX 2(i)

Nitrogen and phosphorus levels in the bark and wood of 2-year-old inoculated limbs at inoculation in the P experiment.

Nitrogen content (% dry matter)

Rep.	P_0 Wood	P_0 Bark	P_1 Wood	P_1 Bark	P_2 Wood	P_2 Bark
1	0.156	0.956	0.176	1.084	0.182	1.040
2	0.208	0.931	0.168	0.988	0.240	1.058
3	0.171	0.746	0.200	1.167	0.174	1.058
4	0.181	0.727	0.179	1.116	0.151	1.100
5	0.139	0.784	0.188	1.052	0.206	1.109
6	0.185	0.928	0.179	1.097	0.189	1.071
7	0.249	0.969	0.176	0.988	0.271	1.160
8	0.230	0.893	0.216	1.058	0.193	1.007
9	0.213	1.065	0.207	1.071	0.185	1.097
10	0.224	0.956	0.191	0.924	0.200	1.058
11	0.171	0.880	0.221	1.071	0.198	1.033
12	0.169	1.026	0.236	1.205	0.194	1.026
Total	2.296	10.861	2.337	12.821	2.383	12.817

Phosphorus content (ppm dry matter)

Rep.	P_0 Wood	P_0 Bark	P_1 Wood	P_1 Bark	P_2 Wood	P_2 Bark
1	225	950	340	1720	460	1970
2	235	1035	325	1480	535	2350
3	185	775	485	2215	465	2125
4	200	725	425	1855	365	2000
5	185	875	385	1810	500	2370
6	225	950	380	1625	420	2100
7	285	930	335	1515	575	2505
8	200	835	440	1710	390	2000
9	290	1085	390	1935	420	2180
10	230	925	350	1610	390	2010
11	200	875	410	2000	440	1990
12	260	1165	440	2160	440	1900
Total	2720	11125	4705	21635	5400	25500

APPENDIX 2(j)

The phosphorus content (ppm d.m.) of 2- and 3-year-old wood of uninoculated trees at four times during the 1970-71 season.†

		October 26, 1970	December 6, 1970	February 3, 1971	April 5, 1971
2-year-old wood	P_0	181	124	178	176
	P_1	344	262	324	314
	P_2	352	262	344	299
3-year-old wood	P_0	154	126	186	147
	P_1	303	269	279	300
	P_2	324	249	300	296

† Each figure represents the average of values for two replications.

APPENDIX 2(k)

Levels of soluble sugars, starch and hemicelluloses (mg./g. dry matter) in the bark and wood of the 2-year-old inoculated limbs at inoculation in the P experiment.

Wood

Rep.	P_0 Soluble sugars	Starch	Hemicelluloses	P_1 Soluble sugars	Starch	Hemicelluloses	P_2 Soluble sugars	Starch	Hemicelluloses
1	14.56	34.50	256.2	17.27	35.89	256.2	19.02	35.29	258.7
2	19.02	33.68	260.0	19.15	40.91	248.8	17.27	39.54	247.6
3	13.63	26.56	254.5	17.01	35.24	268.9	13.63	35.68	245.0
4	17.39	29.44	236.2	16.62	34.20	260.1	16.25	33.47	264.2
5	15.88	28.16	242.5	18.19	32.57	242.7	18.19	37.36	260.0
6	14.20	35.54	245.1	18.05	32.60	238.6	17.52	38.58	241.3
7	13.63	29.28	235.2	15.73	32.75	242.3	17.01	37.71	254.5
8	17.65	27.28	254.5	17.92	47.85	247.6	19.85	32.19	254.5
9	20.81	28.07	246.7	16.75	32.23	246.4	17.65	32.56	266.4
10	15.39	32.08	248.8	16.38	33.05	267.6	14.67	40.29	253.2
11	13.19	30.79	254.5	19.15	38.11	265.1	19.15	36.18	260.7
12	13.97	32.56	242.3	18.89	37.98	254.5	17.39	40.65	248.8
Total	189.32	367.94	2976.5	211.11	433.38	3038.8	207.60	439.50	3054.9

Bark

Rep.	P_0 Soluble sugars	Starch	P_1 Soluble sugars	Starch	P_2 Soluble sugars	Starch
1	(1.54	51.30	69.87	38.96	72.78	40.12
2	50.11	41.14	62.08	43.91	59.40	35.14
3	58.85	40.60	63.72	45.94	62.63	46.35
4	58.31	34.64	71.59	45.35	75.15	50.20
5	57.80	43.28	72.20	37.70	72.78	40.20
6	52.08	46.72	58.31	34.98	72.20	41.29
7	47.61	41.74	67.02	41.12	69.29	52.79
8	61.54	46.32	71.02	46.64	73.96	51.80
9	53.12	36.80	66.88	36.06	64.81	33.93
10	49.12	36.14	73.36	40.36	83.12	40.09
11	59.40	38.20	69.81	48.43	60.45	40.08
12	58.31	35.08	70.44	43.82	62.63	44.46
Total	667.79	491.96	816.36	503.87	829.20	515.45

APPENDIX 2(1)

Analysis of variance of the results on which Table 28 is based.

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Replications	11	7957.39	723.39	<1 n.s.
Treatments (T)	2	901.09	450.54	<1 n.s.
Error	22	21564.17	980.18	
Total	35	30422.65		
Var. between pos. of meas. (P) within treatment	18	11565.97	642.56	16.94***
P	6	10067.09	1677.85	44.22***
P x T†	12	1498.88	124.91	3.29***
Error	198	7512.52	37.94	
Total	251	49501.14		

† See Appendix 2(f).